NRA Special Review of

MACROCYCLIC LACTONES

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FOREWORD

The National Registration Authority for Agricultural and Veterinary Chemicals (NRA) is an independent statutory authority with responsibility for the regulation of agricultural and veterinary chemicals.

The NRA's Special Review Program examines agricultural and veterinary chemicals registered in the past to determine whether they continue to meet current standards for registration. Chemicals for review are chosen when there is reason to believe the current conditions for registration are no longer met.

In undertaking reviews, the NRA works in close cooperation with advisory agencies including the Department of Health and Family Services (Chemicals and Non-Prescription Drug Branch), Environment Australia (Risk Assessment and Policy Section), National Occupational Health & Safety Commission (Chemical Assessment Branch) and State Departments of Agriculture.

The NRA has a policy of encouraging openness and transparency in its activities and community involvement in decision-making. The publication of evaluation documents for all reviews is a part of that process.

The NRA also makes these reports available to the regulatory agencies of other countries as part of bilateral exchange agreements and as part of the OECD *ad hoc* exchange program. Under this program it is proposed that countries receiving these reports will not utilise them for registration purposes unless they are also provided with the raw data from the relevant applicant.

This report provides details of the review of macrocyclic lactones that has been conducted by the NRA and its advisory agencies. The review's findings are based on information collected from a variety of sources, including data packages and information submitted by registrants, information submitted by members of the public, key user/industry groups and government organisations, and literature searches.

The information and technical data required by the NRA to review the safety of both new and existing chemical products must be derived according to accepted scientific principles, as must the methods of assessment undertaken. Details of required data are outlined in various NRA publications.

Other publications explaining the NRA's requirements for registration can also be purchased or obtained by contacting the NRA. Among these are: *Ag Manual: The Requirements Manual for Agricultural Chemicals; Vet Manual: The Requirements Manual for Veterinary Chemicals* and Volume II of *Interim Requirements for the Registration of Agricultural and Veterinary Chemical Products.*

CONTENTS

ABBREVIATIONS AND ACRONYMS WHICH APPEAR IN THIS DOCUMENT

Abm	Abamectin
ai	Active Ingredient
CSIRO	Commonwealth Scientific and Industrial Research Organisation
d	Day
h	Hour
i/m	Intramuscular
Ivm	Ivermectin
kg	Kilogram
LC90	Concentration of chemical that kills 90% of the test population of organisms
m	Metre
mg	Milligram
mg	Microgram
mL	Millilitre
Mox	Moxidectin
mth	Month
ppb	Parts per Billion
ppm	Parts per Million
ppt	Parts per Trillion
s/c	Subcutaneous
$t_{1/2}$	Half-life
WHP	Withholding Period
wk	Week

SUMMARY

The NRA has reviewed the registrations of the macrocyclic lactones: ivermectin, abamectin, moxidectin, doramectin and milbemycin in terms of the effects of these products on dung insects and dung degradation in Australia, and label claims in relation to these effects. Particular emphasis was placed on coprophagous beetles originally introduced into Australia under the CSIRO Dung Beetle Program to improve dispersal of cattle dung and control pestiferous dung.

Key Outcomes

From this review, the NRA has concluded that there is no clear evidence to indicate that any of the macrocyclic lactone products have a long term detrimental effect on dung beetle populations or dung disappearance rates in the field in Australian conditions, although different products may have differing short term effects, and differing toxicities to dung beetle larvae.

It is also concluded that there is insufficient data to substantiate broadly based claims of safety to dung beetles in Australian field conditions, as currently contained on some labels, but that labels might be able to be varied in such a way as to contain instructions which accurately reflect the data available for a particular product.

Detailed outcomes from the review are shown on page10 of this report.

Technical Evaluations

Use pattern

Macrocyclic lactones are administered to grazing livestock as oral drench, topical and injectable formulations principally to control gastrointestinal nematode parasites but also to assist in the control of several external parasites including buffalo fly, cattle tick and lice. An intraruminal bolus formulation is also available for sheep.

Macrocyclic Lactones excreted in dung

Macrocyclic lactones are almost entirely excreted in faeces but the rate differs considerably between the various commercial compounds due to differences in their lipophilicity and rates of hepatic metabolism and clearance in the bile. Following subcutaneous or topical dosing, ivermectin levels in cattle faeces peak within 2-6 days and negligible or very low levels persist beyond 14 days. Doramectin is excreted at a similar rate to ivermectin. Moxidectin residues are excreted more slowly and persist in cattle faeces for more than 28 days.

Effects on dung beetles and other insects

Treatment of Cattle

Neither ivermectin nor abamectin is toxic to mature egg-laying adults at concentration likely to be found in dung. However, there is increased mortality and impaired development of larvae with sub-lethal effects on the morphology of some species in dung voided within 2-3 weeks of treatment, and increased mortality and delayed reproductive development in newly emerged adults of some species feeding on dung voided within 1 to 2 weeks of treatment. Dung of treated cattle is highly toxic to dipteran larvae, inhibiting development for periods ranging from 2 to 8 weeks. The duration of these toxic effects on dung insects is consistent with the profile of excretion of these avermectins in cattle faeces.

Under European field conditions, treatment with ivermectin or abamectin has little effect on colonisation of cattle dung pats by adult coprophagous beetles but larval numbers are reduced in dung deposited for up to 2-3 weeks after treatment. There is no evidence for long-term adverse effects of ivermectin or abamectin residues on the degradation of dung pats or on the accumulation of dung on pasture, despite initial reductions in insect larvae and dung disappearance rate.

No comparable long-term studies have been undertaken under Australian conditions but it has been shown that adult native and introduced species of dung beetles actively colonise the dung of treated cattle and that short-term increases occur in burial rates of dung containing avermectin residues.

In Australia, dung beetle populations may be exposed to risk from toxic residues of ivermectin and abamectin during larval development and following emergence of immature adults. Quantitation of the overall impact on the population dynamics of native and introduced dung beetles under Australian conditions is not possible at present. Computer models that simulate the biological characteristics of the introduced dung beetle species may assist in identifying the most sensitive elements of risk to these populations and enable livestock treatments to be timed optimally to minimise that risk whilst maintaining a balance between effective parasite control and sustainable beetle populations.

Moxidectin residues in cattle dung are considered non-toxic to egg-laying adults and developing larvae. Under European field conditions, colonisation and larval development of native Coleoptera in dung pats were unaffected by moxidectin residues. Development of dipteran larvae is generally not affected by moxidectin residues in cattle dung although development of hornfly larvae may be impaired. No data are available on the effects of moxidectin residues in cattle dung on mortality of newly emerged adult dung beetles or their reproductive development.

No field studies have been reported under Australian conditions of the impact of moxidectin on dung colonisation by native and introduced beetles or on dung degradation. The limited data available indicate that moxidectin is unlikely to have a negative impact on dung beetle populations but more extensive and longer-term testing against a wider range of introduced species is required to adequately define the risk.

Doramectin may have toxic effects on dung beetles when used in cattle. Residue concentrations high enough to kill an estimated 90% of laboratory populations of larvae is apparently exceeded in cattle faeces for at least 2 weeks after treatment but no data have been submitted to indicate whether doramectin residues in the dung of treated cattle affect any stage of the life cycle of coprophagous beetles. The effects of doramectin residues on insect colonisation or on degradation of dung have not been examined under Australian conditions.

Treatment of Sheep

Limited studies show that oral administration of ivermectin to sheep has some short term effects on larvae and newly emerged adults. These short-term effects are consistent with the rapid excretion of residues in dung, and indicate that other species feeding on sheep dung will be exposed to toxic residues for a short time only.

The recommended use pattern for oral ivermectin drenches in sheep in the summer and winter rainfall zones indicates that native and introduced dung beetle populations will be exposed to ivermectin residues periodically throughout the active breeding cycle. However, the short-term toxicity of these residues suggests that there is a very low risk to sustainability of these populations.

Treatment of sheep with ivermectin continuously for 100 days using an intraruminal bolus increases the exposure risk for both native and introduced species of dung beetles but residue levels in faeces may be below the lethal threshold for introduced dung beetles. Sub-lethal effects on dung beetles of long-term exposure to these low ivermectin residue levels in sheep faeces require evaluation to assess the potential for impact on population sustainability.

Moxidectin residues excreted in sheep dung during the first two days after oral treatment inhibit larval development in the one species of dung beetle tested. This is consistent with the high residue levels in faeces at that time and suggests that other species may be similarly affected. These observations, together with the protracted excretion profile for moxidectin residues in sheep dung, indicate the need to evaluate possible sub-lethal effects on larvae and immature adults of the more important Australian species to assess the risk exposure to dung beetle populations of moxidectin drenching of sheep.

Treatment of Horses

Recommended worm control programs for horses potentially expose dung beetles to toxic levels of ivermectin residues for the first 2-3 days after dosing. No data are available on the influence of treatment on dung colonisation or mortality of beetles under Australian conditions but rapid clearance and consequent short duration of exposure to ivermectin residues in horse dung suggests a low impact on overall beetle populations. A moxidectin based preparation is also registered for use in horses; no data is available regarding the effects of this product on horse dung.

Treatment of Dogs

Monthly prophylactic treatment of dogs with macrocyclic lactones presents an extremely low risk to sustainability of dung beetle populations in view of the low dose rates used coupled with the minor role that dog faeces probably represents as a food resource for these populations.

1. OVERVIEW REPORT

The National Registration Authority for Agricultural and Veterinary Chemicals (NRA) has reviewed the active ingredients, macrocyclic lactones, and all veterinary chemical products containing macrocyclic lactones and associated labels, with a particular focus on the effects of these products on dung beetle populations and dung degradation rates.

The purpose of this document is to provide a summary of the data evaluated and of the regulatory decisions reached, as a result of the Special Review of macrocyclic lactones.

1.1 Regulatory Information

Initiating a Special Review

The NRA has statutory powers to reconsider the approval of active constituents, the registration of chemical products or the approval of labels for containers at any time. The basis for a reconsideration is whether the NRA is satisfied that the requirements prescribed by the Agricultural and Veterinary Chemicals Code (scheduled to the *Agricultural and Veterinary Chemicals Act 1994*) for continued approval are being met. These requirements are that the use of an active constituent or product, in accordance with the recommendations for its use:

- would not be an undue hazard to the safety of people exposed to it during its handling or people using anything containing its residues;
- would not be likely to have an effect that is harmful to human beings;
- would not be likely to have an unintended effect that is harmful to animals, plants or things or to the environment; and
- would not unduly prejudice trade or commerce between Australia and places outside Australia.

Obligations to submit data and other information on chemicals under Special Review

On initiating a review, the NRA has to notify relevant approval holders and registrants of the matters it intends to reconsider and its reasons for doing so, and to invite them to make written submissions on those matters. These parties are also requested to submit all existing information and data (regardless of its age or confidentiality) on the chemical under review.

The NRA may also consult with the members of the community and persons, organisations or government agencies with relevant knowledge or interests for the purposes of obtaining information or advice relating to the review.

Once a review is under way, the NRA may request additional information from approval holders and registrants. If such a request is denied, the NRA may suspend or cancel the relevant approval or registration.

Outcomes of reviews

There are three possible outcomes to an ECRP review:

1. The NRA is satisfied that the chemical under review continues to meet the prescribed requirements for the initial approval or registration and confirms the approval or registration.

2. The NRA is satisfied that the conditions to which the approval or registration is currently subject can be varied in such a way that the requirements for continued approval or registration will be complied with and varies the conditions of approval or registration.

3. The NRA is not satisfied that the conditions continue to be met and suspends or cancels the approval or registration.

The NRA must notify the approval holders and registrants of the outcomes of these reviews.

1.2 Data Protection

To grant protection to providers of certain information relating to agricultural and veterinary chemicals, the NRA introduced a program of data protection. The objectives of this program are:

- to provide an incentive for the development of products and data applicable to Australian or local conditions;
- to encourage the availability of overseas products and data; and
- to provide reciprocal protection for Australian products and data under overseas' data protection systems.

In general, the NRA designates information as 'protected information' for a 'protection period' of two to seven years if the information:

- is requested by the NRA for the purposes of reviewing a product;
- is relevant to the scope of the review; and
- relates to the interaction between the product and the environment of living organisms or naturally occurring populations in ecosystems, including human beings.

If the NRA proposes to use the same information to determine whether to register, or continue registration, of another chemical product, the NRA must not use the information until the parties come to an agreement as to terms for compensation, unless the protection period has expired or the NRA is satisfied that it is in the public interest to use the information.

The studies designated as having protected status are marked with a "P" in the list of references of the detailed technical report.

1.3 Chemical and Product Details

History of Registration

Macrocyclic lactones registered for use in Australia comprise the avermectins (ivermectin, abamectin and doramectin) and the milbemycins (milbemycin oxime and moxidectin). Ivermectin was the first of the MLs to be developed. It was first marketed in 1981 as an anti-parasite drug and first registered in Australia in the mid-1980s. MLs are now widely used around the world with registrations in over 60 countries, including Canada, many European countries, India, Argentina, Japan, New Zealand, Zimbabwe and United States of America.

Doramectin was first registered in Australia in 1996, and moxidectin in 1994.

Details of all products covered by this review are at Attachment 1.

Use Pattern

The MLs are primarily used to control internal parasites such as nematodes and external pests such as insects, ticks and mites in cattle, sheep, pigs, horses, dogs and cats.

Benefits of Macrocyclic Lactones

Drugs that control internal and external parasites of cattle and sheep are essential to the economic productivity of modern, intensive livestock grazing and important to maintain the welfare of domestic livestock and companion animals. These parasites can and do develop resistance to drugs and, in the past decade, several new classes of broad spectrum chemicals have been introduced to the Australian market, including the macrocyclic lactones, which are now widely used in this country and around the world.

Products containing MLs are important for the treatment and prevention of animal diseases caused by nematodes, insects, ticks and mites because of the their high degree of efficacy and broad spectrum action at low doses. Their actions on parasites affect nerve transmission processes that are either not present in mammals or occur in body compartments to which therapuetic doses do not readily distribute. The unique mode of action of MLs provides large margins of safety for host animals and provides valuable efficacy against parasites resistant to other compounds.

1.4 Reasons for the Macrocyclic Lactone Review

The role of coprophagous beetles in the complex process of degradation of animal faeces is considered an important issue for the stability and sustainability of Australia's natural environment, in both rural and urban contexts.

ML compounds are excreted by animals mainly in the faeces. Because of their properties, they have the potential to affect invertebrates that may utilise animal dung as a food or breeding resource. This potential was recognised during the original registration process for ML products and the NRA evaluated data relating to the effects of drug residue in faeces on dung-dwelling invertebrates. As toxicity to larvae was transient and there were few noticable effects on adult insects, the NRA concluded at that time that, under Australian management conditions, treatment of animals (particularly grazing livestock) did not pose an unacceptable risk to dung-dwelling insects.

However, over the past decade concerns have been increasingly expressed by the scientific community and the general public regarding possible unintended side effects of chemicals used in veterinary and agricultural practice and, in particular, the widespread use of the macrocyclic lactone class of chemicals principally as anthelmintics to control gastrointestinal parasites of grazing livestock and companion animals.

There is also a lack of consensus in the scientific community over the potential of residues of macrocyclic lactones, excreted in the dung of treated animals, to harm dung beetles that utilise this dung. Scientists have found it difficilt to agree on the extent of such risks considering the complex dynamics of insect populations that fluctuate widely due to factors such as temperature changes, rainfall, cattle management, pasture quality and climate. This has raised concerns not only because of the possible impact on dung degradation but also the consequences on stability of pastureland insect communities and ecosystems and the sustainability of pasture fertility. In Australia, such concerns have an added interest because of government-funded programs to import dung beetles, adapted to the dung of large ruminants, with the objective of controlling plagues of the nuisance bushfly *Musca vetussima* and the buffalo fly, *Haemotabia irritans exigua*, (a parasite of cattle) that breed in cattle dung.

Therefore, the macrocyclic lactones were placed under Special Review because the NRA was no longer satisfied that these products, used in accordance with the use patterns appearing on currently approved labels, would not be likely to have an unintended harmful effect on the environment. In particular, the NRA was acting on advice from Environment Australia indicating that data are lacking to unequivocably demonstrate that these chemicals do not have a harmful effect on dung beetle populations.

1.5 Scope of the Review

The overall scope of the review is to evaluate the effects of macrocyclic lactones on dung insect populations and dung degradation in Australia. To achieves this, consideration was given to all published scientific literature, agricultural and veterinary company "in-house" data and other scientific literature that has been subjected to peer review relating to the effects of macrocyclic lactones on dung beetles, associated fauna and dung degradation in Australia. In specific terms, the review aimed to reach objective conclusions with regard to the following:

1. The extent of available information on known short and long term effects of ivermectin, abamectin, moxidectin, doramectin and milbemycin on dung insects and overall dung degradation rates, including the number of studies available as well as an assessment of the number of species tested.

2. The frequency and duration of exposure of these 5 macrocyclic lactone actives under actual Australian use patterns and the likely effects of such exposure on overall populations of dung beetles considering all other factors influencing their population dynamics.

3. The overall effect on dung degradation rates and pasture quality resulting from the use of this class of compounds considering the many other factors affecting dung degradation (such as climate, weather, bacteria, fungi and non insect fauna).

4. The effect of diet and management practices on the availability of this class of compounds in the dung and their impact on dung beetles.

1.6 Notification of the Review

All registrants of veterinary products containing macrocyclic lactones were notified of the review, its scope and given the opportunity to provide any information, of which they were aware, that was relevant to the review, particularly any information relating to effects on dung-dwelling fauna. State Departments of Agriculture/ Primary Industries were also asked to comment on the review.

1.7 Responses from Participants in the Review

Extensive submissions of data were made by registrants in response to the review notification, while some information on use of these products was provided by some of the States. This information, together with that available in the published scientific literature, was considered in the review.

1.8 Evaluation of Submissions

The NRA requested an independent scientific expert from the CSIRO to review the data and information of relevance to this review. The full report can be found in Section 2.

The main findings of the review are as follows.

Macrocyclic Lactones excreted in dung

Macrocyclic lactones are almost entirely excreted in faeces but the rate differs considerably between the various commercial compounds due to differences in their lipophilicity and rates of hepatic metabolism and clearance in the bile. Following subcutaneous or topical dosing, ivermectin levels in cattle faeces peak within 2-6 days and negligible or very low levels persist beyond 14 days. Doramectin is excreted at a similar rate to ivermectin. Moxidectin residues are excreted more slowly and persist in cattle faeces for more than 28 days.

Effects on dung beetles and other insects

Treatment of Cattle

Ivermectin or abamectin administered in subcutaneous or topical formulation has the following effects on a range of introduced dung beetle species: no toxicity to mature egg-laying adults; increased mortality and impaired development of larvae with sub-lethal effects on the morphology of some species in dung voided within 2-3 weeks of treatment; increased mortality and delayed reproductive development in newly emerged adults of some species feeding on dung voided within 1 to 2 weeks of treatment. Dung of treated cattle is highly toxic to dipteran larvae, inhibiting development for periods ranging from 2 to 8 weeks. The duration of these toxic effects on dung insects is consistent with the profile of excretion of these avermectins in cattle faeces.

Under European field conditions, treatment subcutaneously or topically with ivermectin or abamectin has little effect on colonisation of cattle dung pats by adult coprophagous beetles but larval numbers are reduced in dung deposited for up to 2-3 weeks after treatment. There is no evidence for long-term adverse effects of ivermectin or abamectin residues on the degradation of dung pats or on the accumulation of dung on pasture, despite initial reductions in insect larvae and dung disappearance rate. No comparable long-term studies have been undertaken under Australian conditions but it has been shown that adult native and introduced species of dung beetles actively colonise the dung of treated cattle and that short-term increases occur in burial rates of dung containing avermectin residues.

Manufacturers' recommendations for usage of ivermectin and abamectin products in cattle in Australia expose dung beetle populations to risk from toxic residues during larval development and following emergence of immature adults. Quantitation of the overall impact of ivermectin and abamectin products for cattle on the population dynamics of native and introduced dung beetles under Australian conditions is not possible at present. Computer models that simulate the biological characteristics of the introduced dung beetle species may assist in identifying the most sensitive elements of risk to these populations and enable livestock treatments to be timed optimally to minimise that risk whilst maintaining a balance between effective parasite control and sustainable beetle populations.

Limited studies with two species of introduced dung beetles show that moxidectin residues in cattle dung are not toxic to egg-laying adults or to developing larvae. Under European field conditions, colonisation and larval development of native Coleoptera in dung pats were unaffected by moxidectin residues. Development of dipteran larvae is generally not affected by moxidectin residues in cattle dung although development of hornfly larvae may be impaired. No data are available on the effects of moxidectin residues in cattle dung on mortality of newly emerged adult dung beetles or their reproductive development.

No field studies have been reported under Australian conditions of the impact of moxidectin on dung colonisation by native and introduced beetles or on dung degradation. Moxidectin is apparently less toxic intrinsically to larvae of one species of introduced dung beetle and to buffalo fly than abamectin. There has been no examination of possible sublethal effects on beetle populations arising from the longer term excretion pattern of moxidectin in cattle dung. The limited data available indicate that moxidectin is unlikely to have a negative impact on dung beetle populations but more extensive and longer-term testing against a wider range of introduced species is required to adequately define the risk.

No data have been submitted to indicate whether doramectin residues in the dung of treated cattle affect any stage of the life cycle of coprophagous beetles although an LC_{90} has been established against the larvae of one introduced species. This concentration is apparently exceeded in cattle faeces for at least 2 weeks after treatment, suggesting that doramectin may have toxic effects on dung beetles when used in cattle. The effects of doramectin residues on insect colonisation or on degradation of dung have not been examined under Australian conditions.

Treatment of Sheep

Limited studies on one species of introduced dung beetle show that oral administration of ivermectin to sheep has no effect on adult mortality, has short-term effects on development of larvae, and increases mortality and impairs reproductive development of newly emerged adults in dung voided 1-2 days after treatment. These short-term effects are consistent with the rapid excretion of residues in dung, and indicate that other species feeding on sheep dung will be exposed to toxic residues for a short time only.

The recommended use pattern for oral ivermectin drenches in sheep in the summer and winter rainfall zones indicates that native and introduced dung beetle populations will be exposed to ivermectin residues periodically throughout the active breeding cycle. However, the short-term toxicity of these residues suggests that there is a very low risk to sustainability of these populations.

Treatment of sheep with ivermectin continuously for 100 days using an intraruminal bolus increases the exposure risk for both native and introduced species of dung beetles but residue levels in faeces may be below the lethal threshold for introduced dung beetles. Faecal accumulation on paddocks was not affected during and after treatment with the bolus, but this does not adequately measure impact on populations of dung beetles because of their minor role in decomposition of sheep dung under Australian conditions. Sub-lethal effects on dung beetles of long-term exposure to these low ivermectin residue levels in sheep faeces require evaluation to assess the potential for impact on population sustainability.

Moxidectin residues excreted in sheep dung during the first two days after oral treatment inhibit the development of larvae of the one species of dung beetle which has been examined. This is consistent with the high residue levels in faeces at that time and suggests that other species may be similarly affected. These observations, together with the protracted excretion profile for moxidectin residues in sheep dung, indicate the need to evaluate possible sub-lethal effects on larvae and immature adults of the more important Australian species in order to assess the risk exposure to dung beetle populations of moxidectin drenching of sheep.

Treatment of Horses

Recommended worm control programs for horses potentially expose dung beetles to toxic levels of ivermectin residues for the first 2-3 days after dosing. No data are available on the influence of treatment on dung colonisation or mortality of beetles under Australian conditions but rapid clearance and consequent short duration of exposure to ivermectin residues in horse dung suggests a low impact on overall beetle populations. No data are available on the moxidectin formulations for use in horses.

Treatment of Dogs

Monthly prophylactic treatment of dogs with macrocyclic lactones presents an extremely low risk to sustainability of dung beetle populations in view of the low dose rates used coupled with the minor role that dog faeces probably represents as a food resource for these populations.

1.9 Conclusions

The detailed technical review of currently available data published in the scientific literature and submitted 'in-house' company reports has shown that there is a need to generate further information to more adequately quantify the risk to dung beetle populations posed by specific compounds within the macrocyclic lactone class. For this reason, the inclusion of broadly based statements on labels which claim that products are safe to dung beetle populations cannot be supported.

The data available do not, however, indicate that long term damage to dung beetle populations in the field is occurring as a result of use of macrocyclic lactones. There is therefore considered to be no need at this stage to require additional information regarding dung beetle safety as a condition of continued registration, although additional work in this regard is to be encouraged.

Additional investigations which would enable a more complete understanding of the effects of macrocyclic lactones on dung beetle populations and dung degradation include:

- a detailed analysis of the risk to the more important introduced species of coprophagous beetles associated with use of ivermectin and abamectin products in cattle in Australia utilising all relevant information on their toxic effects;
- the generation of data on the influence of long-term exposure of dung beetles to residues in faeces during treatment of sheep with the ivermectin controlled release bolus;

- further investigation of toxicity of moxidectin to other genera of introduced dung beetles, particularly *Onitis* spp., as well as sublethal effects of moxidectin residues on larval development, on mortality of newly emerged adults, and on their reproductive development in the more important introduced genera of Australian dung beetles;
- if the latter studies indicate toxic effects on all or some species, modelling the impact of moxidectin treatment on dung beetle populations as well as for ivermectin/abamectin products; and
- generation of a complete data package of the short- and long-term lethal and sub-lethal effects of doramectin residues in cattle dung on the more important introduced species of coprophagous beetles.

However, while the conduct of investigations such as those described above would provide a more detailed understanding of the effect of macrocyclic lactone products on dung beetles, it would not be possible to ever prove conclusively that a product was totally safe to all species in all circumstances.

1.10 Review Outcomes

Based on the findings of the detailed review, it is concluded that there is no clear evidence to demonstrate that any of the macrocyclic lactone products have a long term detrimental effect on dung beetle populations or dung disappearance rates in the field in Australian conditions.

While the different products may have differing short term effects, and exhibit differing toxicities to dung beetle larvae, there is also insufficient long term data to substantiate broadly based claims of safety to dung beetles in Australian field conditions.

Accordingly, the NRA has decided that:

- *on the basis of currently available information, it is satisfied that the continued use of products containing macrocyclic lactones 'would not be likely to have an unintended effect that is harmful to animals, plants or things or to the environment', as required by section 34(1)(a)(iii) of the Agvet Code;*

- *it is not satisfied that current labels for certain macrocyclic lactone products (which contain broad claims of safety to dung beetles) contain adequate instructions as required by section 34(1)(c), or that they comply with the requirements for continued approval according to section 34(1)(d), and that these claims should therefore be disallowed; but that*

    - *pursuant to section 34(5), the labels might be able to be varied in such a way that the labels would contain adequate instructions and that the requirements for continued approval would be complied with. [The NRA would need to be satisfied that any revised label claims were consistent with the evidence considered in this report and adequately supported by data].*

Attachment 1

Currently Registered Products containing the avermectins (ivermectin, abamectin and doramectin) and the milbemycins (milbemycin oxime and moxidectin).

Ivermectin

Product	Registrant
37526 EQUIMEC PASTE	Merial Australia Pty Ltd *
46459 EQUIMEC TUBING LIQUID BROAD SPECTRUM PARASITE CONTROL AGENT FOR HORSES	Merial Australia Pty Ltd *
37539 HEARTGARD 30 IVERMECTIN CHEWABLES (136MCG IVERMECTIN)	Merial Australia Pty Ltd *
37537 HEARTGARD 30 IVERMECTIN CHEWABLES (272MCG IVERMECTIN)	Merial Australia Pty Ltd *
37538 HEARTGARD 30 IVERMECTIN CHEWABLES (68MCG IVERMECTIN)	Merial Australia Pty Ltd *
39540 HEARTGARD 30 PLUS IVERMECTIN/PYRANTEL CHEWABLES (136UG IVERMECTIN & 114MCG PYRANTEL)	Merial Australia Pty Ltd *
39541 HEARTGARD 30 PLUS IVERMECTIN/PYRANTEL CHEWABLES (68UG IVERMECTIN & 57MCG PYRANTEL)	Merial Australia Pty Ltd *
39539 HEARTGARD 30 PLUS IVERMECTIN/PYRANTEL CHEWABLES (272UG IVERMECTIN & 227MCG PYRANTEL)	Merial Australia Pty Ltd *
37531 HEARTGARD 30 TABLETS 136 MCG	Merial Australia Pty Ltd *
37532 HEARTGARD 30 TABLETS 272 MCG	Merial Australia Pty Ltd *
37530 HEARTGARD 30 TABLETS 68 MCG	Merial Australia Pty Ltd *
47059 HEARTGARD FX (IVERMECTIN) CHEWABLES FOR CATS (165MCG SIZE)	Merial Australia Pty Ltd *
47060 HEARTGARD FX (IVERMECTIN) CHEWABLES FOR CATS (55MCG)	Merial Australia Pty Ltd *
45695 IVOMEC (IVERMECTIN) ANTIPARASITIC INJECTION FOR PIGS	Merial Australia Pty Ltd *
46177 IVOMEC (IVERMECTIN) PREMIX FOR PIGS	Merial Australia Pty Ltd *
45721 IVOMEC (IVERMECTIN, MSD) POUR-ON FOR CATTLE	Merial Australia Pty Ltd *
46570 IVOMEC ANTIPARASITIC INJECTION FOR CATTLE	Merial Australia Pty Ltd *
46911 IVOMEC BOLUS FOR SHEEP	Merial Australia Pty Ltd *

Ivermectin (cont).

Product	Registrant
48688 IVOMEC MAXIMIZER CONTROLLED RELEASE CAPSULES FOR ADULT SHEEP 40 to 80 KG	Merial Australia Pty Ltd *
48689 IVOMEC MAXIMIZER CONTROLLED RELEASE CAPSULE FOR WEAKER SHEEP 20 to 40 KG	Merial Australia Pty Ltd *
37512 IVOMEC LIQUID FOR SHEEP BROAD SPECTRUM ORAL ANTIPARASITIC SOLUTION	Merial Australia Pty Ltd *
45359 IVOMEC PLUS (IVERMECTIN PLUS CLORSULON) BROAD SPECTRUM ANTIPARASITIC INJECTION FOR CATTLE	Merial Australia Pty Ltd *
40510 IVOMEC RV REDUCED VOLUME DRENCH FOR SHEEP	Merial Australia Pty Ltd *
45623 JETAMEC JETTING FLUID CONCENTRATE	Merial Australia Pty Ltd *
46445 NUFARM ANIMAL HEALTH ORAMEC LIQUID FOR SHEEP	Merial Australia Pty Ltd *
48813 ORAMEC BOLUS FOR SHEEP	Merial Australia Pty Ltd *

Abamectin

Product	Registrant
46234 DUOTIN ANTIPARASITIC INJECTION FOR CATTLE	Merial Australia Pty Ltd *
47652 VIRBAMEC ANTIPARASITIC INJECTION FOR CATTLE	Virbac (Australia) Ltd

Doramectin

Product	Registrant
46128 DECTOMAX INJECTABLE ENDECTOCIDE	Pfizer

Moxidectin

Product	Registrant
45925 PROHART TABLETS FOR LARGE DOGS	Fort Dodge Australia Pty Ltd #
45663 CYDECTIN INJECTION FOR CATTLE	Fort Dodge Australia Pty Ltd #
46517 CYDECTIN LV LOW VOLUME DRENCH FOR SHEEP	Fort Dodge Australia Pty Ltd #
45738 CYDECTIN ORAL DRENCH FOR SHEEP	Fort Dodge Australia Pty Ltd #

Moxidectin (cont.)

Product	Registrant
45925 PROHART TABLETS FOR LARGE DOGS	Fort Dodge Australia Pty Ltd #
45970 CYDECTIN POUR-ON FOR CATTLE AND RED DEER	Fort Dodge Australia Pty Ltd #
47339 PROHART TABLETS FOR MEDIUM DOGS	Fort Dodge Australia Pty Ltd #
47340 PROHART TABLETS FOR SMALL DOGS	Fort Dodge Australia Pty Ltd #

Milbemycin Oxime

Product	Registrant
40109 ENDOVET ANTHELMINTIC TABLETS FOR LARGE DOGS	Novartis Animal Health Australasia Ltd
40110 ENDOVET ANTHELMINTIC TABLETS FOR MEDIUM DOGS	Novartis Animal Health Australasia Ltd
40112 ENDOVET ANTHELMINTIC TABLETS FOR VERY SMALL DOGS	Novartis Animal Health Australasia Ltd
40111 ENDOVET ANTHELMINTIC TABLETS FOR SMALL DOGS	Novartis Animal Health Australasia Ltd
47457 ENDOVET FLAVOUR TABS FOR LARGE DOGS	Novartis Animal Health Australasia Ltd
47456 ENDOVET FLAVOUR TABS FOR MEDIUM DOGS	Novartis Animal Health Australasia Ltd
47455 ENDOVET FLAVOUR TABS FOR SMALL DOGS	Novartis Animal Health Australasia Ltd
47454 ENDOVET FLAVOUR TABS FOR VERY SMALL DOGS	Novartis Animal Health Australasia Ltd
49474 INTERCEPTOR FLAVOUR TABS FOR VERY SMALL DOGS	Novartis Animal Health Australasia Ltd
49475 INTERCEPTOR FLAVOUR TABS FOR SMALL DOGS	Novartis Animal Health Australasia Ltd
49476 INTERCEPTOR FLAVOUR TABS FOR MEDIUM DOGS	Novartis Animal Health Australasia Ltd
49477 INTERCEPTOR FLAVOUR TABS FOR LARGE DOGS	Novartis Animal Health Australasia Ltd
48851 SENTINEL TABLETS FOR VERY SMALL DOGS	Novartis Animal Health Australasia Ltd
48852 SENTINEL TABLETS FOR SMALL DOGS	Novartis Animal Health Australasia Ltd
48853 SENTINEL TABLETS FOR MEDIUM DOGS	Novartis Animal Health Australasia Ltd
48854 SENTINEL TABLETS FOR LARGE DOGS	Novartis Animal Health Australasia Ltd

* formerly Merck Sharp & Dohme (Australia) Pty Ltd

\# formerly Cyanamid Webster Animal Health & Nutrition and Cyanamid Agriculture Pty Ltd

2. DETAILED TECHNICAL REPORT

Assessment of the Effects of the Macrocyclic Lactone Class of Chemicals on Dung Beetles and Dung Degradation in Australia

[Report by Dr John W Steele, CSIRO Division of Animal Production]

Contents

1. INTRODUCTION

The overall purpose of this report is to consider all published scientific literature, agricultural and veterinary company "in-house" data and other scientific literature that has been subjected to peer review relating to the effects of macrocyclic lactones on dung beetles, associated fauna and dung degradation in Australia. The report aims to reach objective conclusions with regard to the terms of reference of the review of the macrocyclic lactones.

2 COLONISATION AND DECOMPOSITION OF HERBIVORE DUNG

Herbivore dung consists principally of water (65-90%), undigested and finely divided plant remains, secretory and excretory products from the gut, bacteria, yeasts, fungi and other micro-organisms and their metabolic products, together with cellular debris from the gut mucosa. Small changes in moisture content can have marked impact on the colonisation and utilisation of dung by insects (Barth, Karrer & Heinze-Mutt, 1995) and most insect feeding occurs in relatively fresh dung within a week or two after deposition, with most of the colonising activity occurring in the first 24 to 96 hours (MacQueen, 1975; Ridsdill-Smith, 1993). The size of a dung pat, or dropping, is correlated with its lifespan as a suitable microhabitat; small droppings dry out faster than large ones and quickly become unsuitable for dung-inhabiting insects (Hanski, 1991). Large herbivore droppings, such as cattle dung, are colonised by a succession of biotic components comprising a large number of organisms such as fungi, bacteria, nematodes and earthworms, in addition to a diverse, complex and interactive insect community. Under temperate European conditions the cattle dung habitat characteristically provides a complex food-web with competitive, interactive elements between the insect components depending on their position in the food chain (see Fig. 1). The taxonomic composition of beetles and flies feeding and breeding in this dung microhabitat can be categorised according to their food-web position as coprophages, mycophages, saprophages, predators or parasitoids (Table 2.1).

The principal focus of this review is the coprophagous insect fauna of cattle dung in Australia which by comparison with Europe contains an impoverished fauna and is used primarily by several species of abundant flies and 20 or so purposely introduced dung beetles together with a few native dung beetle species which have adapted successfully to utilising the dung of introduced livestock. Introduction of exotic species of dung beetles was undertaken by CSIRO initially to cope with the accumulation of cattle dung pats on pasture, particularly in northern Australia where pats could survive intact for a year or more (Ferrar, 1975), but also to control two pestiferous Diptera, the native bush fly *Musca vetustissima* and the introduced buffalo fly *Haematobia irritans exigua* (Waterhouse, 1974). In southern Australia, whilst indigenous dung beetles utilise and bury significant quantities of cattle dung (Hughes, 1975), exotic beetles were introduced to improve dung dispersal and effect better control of the bushfly in this region.

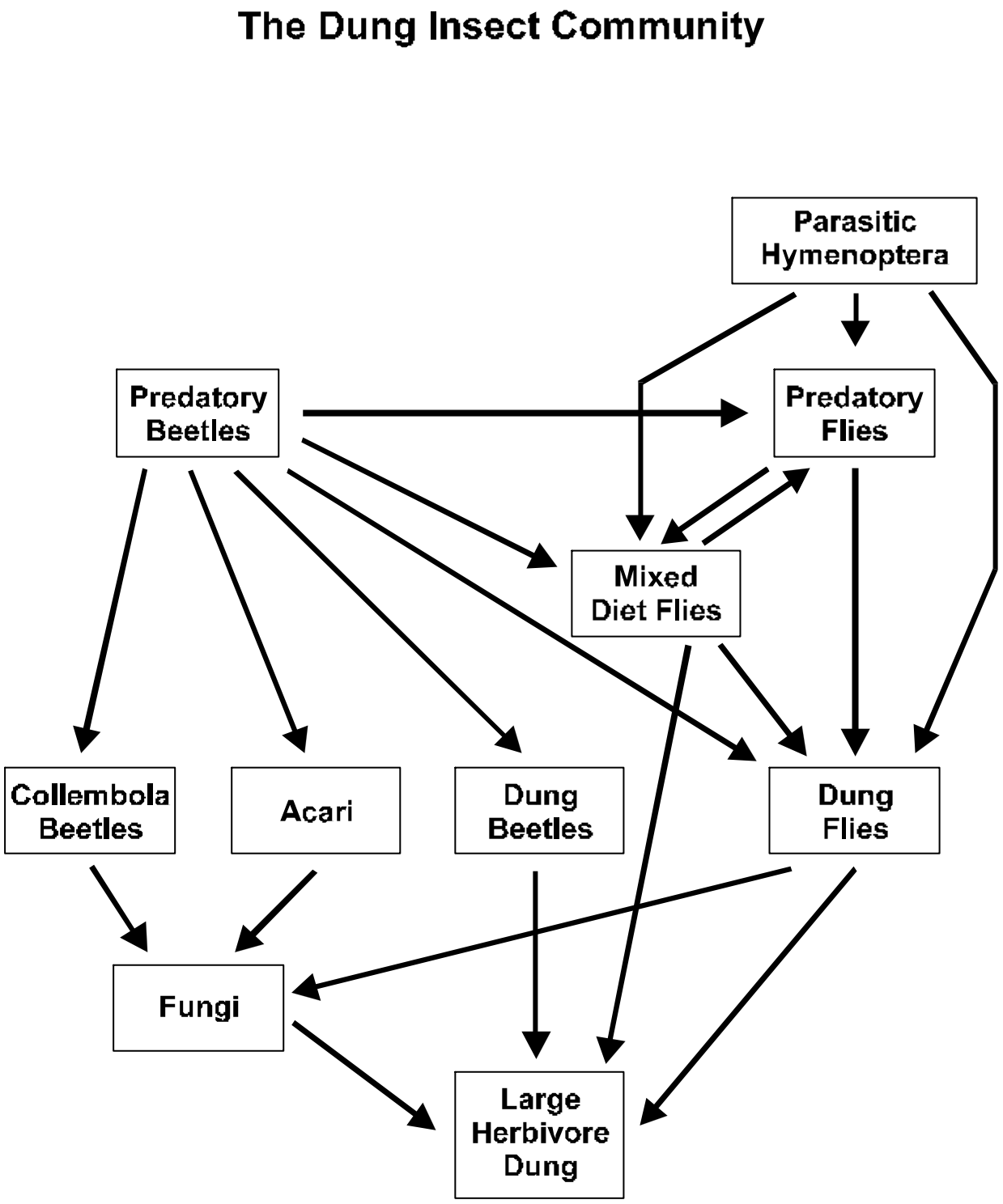

Fig. 1. A simplified food web of the insect community inhabiting cattle dung in Europe (from Hanski 1991).

Table 2.1 Insect families with taxa feeding and/or breeding in the dung microhabitat

Food-Web Position	Coleoptera	Diptera	Hymenoptera
Coprophages	**Aphodiidae** **Geotrupidae** **Hydrophilidae** **Scarabaeidae** **Staphylinidae**	Anthomyiidae Ceratopogonidae Chironomidae **Muscidae** Psychodidae **Scatophagidae** Scatopsidae Sciaridae **Sepsidae** **Sphaeroceridae** Stratiomyidae	
Mycophages	Cryptophagidae Ptiliidae		
Saprophages	**Staphylinidae**		
Predators	Carabidae **Histeridae** **Hydrophilidae** **Staphylinidae**	Muscidae	
Parasitoids	Staphylinidae	Bombyliidae	**Braconidae** **Eucoilidae** Ichneumonidae Pteromalidae

Note: Taxa that are most often numerically or functionally dominant are in boldface.
Source: Hanski, 1991.

Dispersal and degradation of cattle and other herbivore dung by coprophagous beetles is achieved by various mechanisms but there are essentially three behavioural or functional groups that can be discerned, namely, the dwellers, the tunnelers, and the rollers (see Cambefort & Hanski, 1991). The relatively small *Aphodiidae* comprise the bulk of dwellers that eat their way through the dung and deposit eggs without constructing a nest or chamber. *Geotrupidae* and many tribes of *Scarabaeidae* are tunnelers which dig below the dung pat and transport dung to the bottom of the burrow to be used for adult feeding or for breeding. The rollers, comprising many *Scarabaeidae*, construct a dung ball and roll it for a distance before burying it. Adult beetles also disrupt fresh dung through their feeding activities on the fluid constituents, obtained by grinding and pressing the fibrous constituents which are then rejected, leaving shredded dry flakes (Ridsdill-Smith, 1993). Newly hatched dung beetle larvae have well-developed chewing mouth parts and feed on the almost dry dung within the brood ball until emergence (MacQueen, 1975). On the other hand, coprophagous dipteran larvae have no mechanism for chewing and ingest fluids as they move through the dung physically disrupting the pat and assisting its desiccation.

Compared to cattle dung, sheep dung is apparently a less favourable habitat for invertebrates because of its smaller surface area in contact with the soil (King, 1993). By virtue of their structure and small volume, sheep dung pellets dry quickly and are unlikely to remain attractive to insects for more than a day, except perhaps in winter (Wardhaugh *et al*., 1993). Although dung beetles are generally considered to be a less important component of the biota responsible for decomposition of sheep dung, there are clear associations between the abundance of beetles and availability of droppings on sheep pastures (King, 1993; Doube & Wardhaugh, 1991).

Whilst recognising the substantial role of coprophagous beetles and flies in the initial degradation process, one should also note that there are many other biotic and abiotic facilitators and factors that participate in the ultimate dispersal of herbivore dung and its incorporation into the underlying soil. These have been summarised by Forbes (1996) as follows:

Process	Facilitators/Factors
Dehydration	Initial water content Ambient temperature Rainfall Surface area
Fragmentation and transport	Adult dung beetles Earthworms Endocoprid insect larvae Insectivorous birds and mammals Trampling by livestock
Digestion and decomposition	Dung beetle larvae Earthworms Other soil invertebrates Soil associated fungi and bacteria

3. INDIGENOUS AND INTRODUCED DUNG BEETLES IN AUSTRALIA

The geographical distribution, habitat associations, seasonal activities and abundance of indigenous and exotic dung beetles in tropical, sub-tropical and temperate grasslands of Australia have been comprehensively reviewed by Doube, MacQueen, Ridsdill-Smith & Weir (1991). This report has been used as a principal source for a summary of factors considered relevant to understanding exposure risk and likely impacts on Australian dung beetle populations from the use of macrocyclic lactones on domestic livestock.

The indigenous species of dung beetles are well-adapted to using the comparatively dry fibrous faecal pellets of the native marsupials for food and nesting material, rather than the larger, moister dung pads of cattle. The biology and ecology of these species are not well understood although they are known to be restricted largely to forest and woodland habitats. However, some indigenous *Onthophagus* species are found in open grasslands of northern, central and southern Australia where they use dung of sheep, cattle and horses. The indigenous fauna includes 315 species belonging to 20 genera of the family *Scarabaeidae*, 127 species of *Aphodiidae*, 40 species of *Hybosoridae*, and 1 species of *Geotrupidae*. Most of these species are found in the higher rainfall zone, although a few occur in the dry interior also. The seasonal pattern of adult activity is generally restricted to the moist seasons with minimal activity during dry periods and there may be profound differences in abundance from year to year at the same site. These fluctuations can be explained by drought and associated changes in the nutritional quality of dung.

Commencing in 1968, the CSIRO Dung Beetle Program imported 52 species of dung beetles from southern Africa and Europe of which a total of 42 species was released and 22 of these had established breeding populations in the field by 1991 (Table 3.1). Some species now occur over much of the continent whereas others are restricted to either the northern (summer rainfall) or southern (winter rainfall) regions.

Table 3.1 Numbers of introduced and established dung beetle species in mainland Australia and Tasmania

Family	Genus	Number of Species	
		Introduced	Established
Geotrupidae	Geotrupes	1	1
Scarabaeidae	Onthophagus	10	5
	Onitis	11	6
	Cheironitis	1	-
	Bubas	1	1
	Liatongus	1	1
	Euoniticellus	4	4
	Copris	6	1
	Neosisyphus	5	3
	Allogymnopleurus	1	-
	Canthon	1	-

Source: Doube *et al.*, 1991

Table 3.2 Species of introduced dung beetles established in the summer and winter rainfall zones of Australia

Rainfall Zone	
Summer	Winter
Onthophagus gazella*	Onthophagus binodis*
Onitis alexis*	Onthophagus taurus
Onitis virudulus*	Onitis alexis*
Onitis pecuarius	Onitis aygulus
Euoniticellus intermedius*	Onitis caffer
Liatongus militaris*	Euoniticellus fulvus
Neosisyphus spinipes*	Euoniticellus pallipes
Neosisyphus rubrus*	Euoniticellus intermedius*
Neosisyphus infuscatus	Bubas bison
Copris elphenon	

*Abundant in particular rainfall zone.
Source: Doube *et al.*, 1991

Release of the first species took place in coastal Queensland near Townsville, Rockhampton and Brisbane where subsequent monitoring showed that 7 species have become abundant and widespread (Table 3.2). In this summer rainfall zone adults of all species are relatively common during the warmer months and scarce during the cool, dry winter period of July and August. Breeding by the tunnelers, *O. gazella* and *E. intermedius*, occurs primarily in spring, summer and early autumn. Larvae develop to third instars during the winter and early spring and pupation and adult emergence occurs in response to increasing spring temperatures, although adequate soil moisture is also a determinant of emergence. The level of dung dispersal

corresponds with seasonal changes in beetle activity, and generally with the level of reproductive activity in terms of number of brood balls produced; dung pat shredding and burial are maximal from December to March.

Introduction of exotic dung beetles to the winter rainfall zone of south western Australia was particularly aimed at reducing the abundance of the bush fly, *M. vetustissima*. These introductions have increased both the level of dung dispersal and the period over which it occurs in summer and autumn, resulting in a shortening of the bush fly season (see Doube *et al.*, 1991). Of the 14 species introduced, at least 9 have become established, with 3 generally being most abundant (Table 3.2). *O. binodis* and *E. pallipes* are active during the dry summer and autumn months and although soil moisture does not affect seasonal activity it is likely to affect their abundance. Ridsdill-Smith (1993) showed that in the winter rainfall region of Western Australia, feeding by newly emerged adult *O. binodis* resulted in shredding of cattle dung between December and May, whereas production of brood masses and eggs resulted in dung burial from September to November. Newly emerged adults of the native species, *O. ferox*, buried dung for feeding in May and June and for brood masses in which to lay eggs between September and November (Ridsdill-Smith, 1993).

Most of the introduced dung beetles in Australia are adapted to cattle dung and together with indigenous species are less abundant in sheep pastures, although some 22 species including 7 exotic *Scarabaeidae* have been associated with sheep faeces in surveys conducted in eastern Australia (Doube & Wardhaugh, 1991; Wardhaugh *et al*., 1993). Geographical distribution of a species is generally determined by climatic tolerances provided that suitable habitat and food resources are available. *O. taurus* and *O. binodis* are common in the south-eastern and south-western winter rainfall zone but are scarce in the warmer summer rainfall zone. By contrast, *E. intermedius* has broad climatic tolerance and has established over most of mainland Australia, whereas *O. gazella* and *S. spinipes* are restricted to summer rainfall regions.

Reproductive performance of many species of dung beetles has been found to vary with the seasonal changes in dung quality. In the summer rainfall zone near Rockhampton, Queensland, more broods were produced by *E. intermedius* feeding on dung voided by cattle grazing during the summer wet season than on the same pasture in the dry winter (MacQueen, Wallace & Doube, 1986). Similarly, in the winter rainfall zone of south-western Australia egg production by *O. binodis* and *O. alexis* was high on dung of cattle feeding on green annual and perennial pastures and low on dead annual pasture (Ridsdill-Smith, 1986). Population density above a critical threshold may also influence reproductive performance through a progressive reduction in dung burial and brood production by tunnelers such as *O. binodis* (Ridsdill-Smith, Hall & Craig, 1982).

Wherever the exotic species have become established in Australia there has been a significant increase in cattle dung dispersal through burial and shredding. Dung beetle activity reduces survival of dung-breeding flies and there is also evidence that, at times, intense beetle activity suppresses regional abundance of the bush fly, *M. vetustissima*, and the buffalo fly, *H. irritans exigua*. Dung burial by beetles has been shown to increase the growth of pasture grasses and it is believed there is some improvement in pasture productivity as a consequence (see Doube *et al*., 1991), although McKinney & Morley (1975) calculated that the additional responses attributable to introduced beetles averaged over the whole paddock were not likely to influence animal production since the area of dung burial constituted only a small proportion of the total.

4. PHARMACOKINETICS, TISSUE RESIDUES AND EXCRETION IN FAECES

The macrocyclic lactones are lipophilic compounds of moderate molecular weight (<1000) which, following absorption into the bloodstream, are widely distributed throughout the various body tissues of animals treated orally, subcutaneously or topically. In general, following equilibration, fat is a major site of drug residues but substantial levels are found also in the liver where macrocyclic lactones are metabolised, conjugated and excreted in bile. Excretion in urine is low, generally less than 3% of the dose, with the remainder occurring in faeces. Differences in molecular structure and physico-chemical properties generate differing pharmacokinetic, tissue residue, metabolic and excretion profiles for ivermectin, moxidectin and doramectin which are relevant to their potential impact on dung fauna. The salient elements of *in vivo* behaviour of the three compounds are therefore summarised here for cattle, sheep and horse formulations.

4.1 Cattle and Sheep

4.1.2 Plasma

Table 4.1 summarises some relevant published data on the pharmacokinetics of ivermectin in plasma of cattle and sheep following administration of the respective commercial formulations. In cattle, peak concentrations are attained between 1 and 3 days of administration by the subcutaneous and topical routes, whereas in sheep a lower maxima is reached in less than 1 day. Biological half-life of elimination is less than 3 days for the oral formulation used for sheep, similar to the intrinsic half-life (2.7 days) of the active compound when administered intravenously, but formulation in organic solvents extends the half-life in cattle of ivermectin given subcutaneously (see Lo *et al.*, 1985) to 8.3 days.

Table 4.1. Pharmacokinetics of ivermectin in plasma following administration of commercial formulations at 200 ug/kg subcutaneously (S/c) and topically to cattle and orally to sheep and horses

Parameter	Cattle			Sheep[3]	Horses[3]
	S/c[1]	S/c[2]	Topical[2]		
Peak concentration (ppb)	44	46	33	22	82
Peak time (h)	24	72	48	16.4	3.3
Biological half-life (d)	8.3	n/a	n/a	2.5	2.8

Sources: [1]Fink & Porras, 1989; [2]Herd, Sams & Ashcroft, 1996;
[3]Marriner, McKinnon & Bogan, 1987

Comparable summary data are given in Table 4.2 for the pharmacokinetics of total moxidectin residues in plasma of cattle and sheep following administration of the commercial formulations at 200 ug/kg. These data suggest that peak levels of moxidectin in cattle may be higher than those of ivermectin (cf Table 4.1) but other measurements of total residues following subcutaneous injection with 300 ug ivermectin/kg recorded peak concentration in plasma of 133 ppb (Chiu *et al.*, 1990) so that there is probably only a marginal difference in maximal levels of the two compounds. Following subcutaneous injection of moxidectin at 200 ug/kg, Miller, Oehler & Scholl (1994) recorded a peak moxidectin concentration of 75 ppb at 4-6 hours and a biological half-life of approximately 1 day whereas Alvinerie *et al.* (1995) reported a peak concentration of approximately 20 ppb moxidectin at 36 hours and a much longer biological half-life of 25.4 days. In the latter study analytical techniques had a detection limit of 0.1 ppb compared to 5 ppb obtainable by Miller *et al.* (1994) and Cyanamid Websters (1996a).

Table 4.2. Pharmacokinetics of total moxidectin residues in plasma following administration at 200 ug/kg of commercial formulations subcutaneously (S/c) and orally to cattle and sheep

Parameter	Cattle	Sheep

	S/c	S/c	Oral
Peak concentration (ppb)	76	19 (15*)	(12*)
Peak time (h)	8	7	8
Biological half-life (d)	3.2	4.4	0.8

(*) Moxidectin level
Sources: Cyanamid-Websters, 1996a,b.

Peak concentrations of moxidectin in plasma of sheep are substantially lower than in cattle, when given either as the subcutaneous or the oral formulation, but occur at a similar time after administration (Table 4.2). Biological half-life of moxidectin is similar in plasma of sheep and cattle following subcutaneous injection, but is substantially shortened when given by oral drench. Because of its highly lipophilic properties moxidectin is rapidly removed from the blood stream into fat depots and this very short plasma half-life may reflect this process rather than the longer term clearance of moxidectin associated with excretion once equilibration throughout the tissue pools has been established.

The intrinsic half-life in plasma of doramectin following intravenous administration to cattle is 3.7 days compared to 2 days for dihydroavermectin B_{1a} the major constituent of ivermectin (Goudie *et al*., 1993). Similarly to ivermectin, the pharmacokinetic behaviour of doramectin in plasma of cattle following subcutaneous injection can be substantially altered through formulation. Wicks *et al*. (1993) showed that formulations based on sesame oil/ethyl oleate mixtures lowered the peak concentration and extended the residence time of doramectin in plasma compared to an aqueous micelle formulation. Table 4.3 summarises the pharmacokinetics of doramectin given to cattle by subcutaneous injection of the commercial oil-based formulation. As might be anticipated from the resemblance in structure and formulation, doramectin behaves similarly to ivermectin with comparable l values for the peak concentration and its timing, although the data indicate a broader based peak for doramectin and ashorter biological half-life than for ivermectin. However, recent comparisons in calves of injectable formulations of the two compounds under the same conditions have shown that elimination rate is slower and mean residence time in plasma is longer for doramectin than ivermectin (Lanusse *et al*., 1996; Toutain, Terhune & Upson, 1996).

Table 4.3. Pharmacokinetics of total doramectin residues and parent drug in plasma of cattle following subcutaneous injection in commercial formulation at 200 ug/kg

Parameter	Total residues[1]	Doramectin [1]	Doramectin[2]
Peak concentration (ppb)	62	43	28
Peak time (h)	24 - 72	24 - 72	72-144
Biological half-life (d)	5.9	6.2	6.6

Sources: [1] Magonigle & Lynch, 1992; [2] Nowakowski *et al.*, 1995.

4.1.2 Tissues

Total residue levels in liver, bile and fat of cattle determined up to 28 days after subcutaneous injection of ivermectin and moxidectin are presented in Table 4.4. Although initial (7 days) residues of ivermectin are higher in liver because of the higher dosage (300 ug/kg) used in these studies, they also reflect the more rapid hepatic uptake and metabolism of ivermectin than of moxidectin. Similarly, the respective half-lives show that ivermectin and its metabolites are more rapidly depleted from liver than moxidectin residues. By 28 days only trace amounts of ivermectin metabolites continue to be excreted in bile whereas 40 ppb of moxidectin residues are present at this time. Similarly, in fat tissue levels of moxidectin residues are substantially higher at 7 days than ivermectin residues, and a depletion rate that is almost half that of ivermectin maintains high levels of the moxidectin 28 days after dosing. Ivermectin is metabolised by fat tissue as well as by the liver (Chiu & Lu, 1989), whereas moxidectin apparently undergoes minimal biotransformation while stored in fat depots (Hayes, 1994; Zulalian *et al.*, 1994). Thus, 28 days after dosing only 19% of ivermectin residues in fat are present as parent drug whereas with moxidectin the parent compound accounts for up to 90% of total residues in fat at this time.

Table 4.4. Total residue levels in liver, bile and fat of cattle dosed subcutaneously with ivermectin (300 ug/kg) or moxidectin (200 ug/kg)

Tissue	Drug	Days after dosing				Depletion half-life (d)
		7	14	21	28	
		(ppb)				
Liver	IVM	782	55	68	11	4.8
	MOX	109	77	n/a	31	11.4
Bile	IVM	273	54	22	1	
	MOX	159	82	n/a	42	11.3
Fat	IVM	270	83	69	29	7.6
	MOX-omental	898	636	n/a	275	12.2
	MOX-back	495	424	n/a	186	14.3

n/a: Not available
Sources: Chiu & Lu, 1989; Zulalian *et al.*, 1994.

Similar patterns are evident in sheep from a comparison of total residue levels in liver, bile and fat following an oral dose of ivermectin or moxidectin (see Table 4.5). Depletion half-life of residues of oral ivermectin in liver and fat of sheep are considerably shorter than for the subcutaneous formulation in cattle and, as in the latter, removal of ivermectin residues from sheep tissues is substantially faster than for moxidectin. Consequently, by 21 days only trace amounts of ivermectin residues are excreted in the bile of sheep whereas significant quantities of moxidectin are still present in bile at 28 days after dosing. Moxidectin is retained in sheep fat depots even longer than in cattle, with a half-life of up to 27 days (see Hayes, 1994) and at 28 days 92% of total residue in fat is present as parent moxidectin. By comparison, 7 days after oral administration of ivermectin to sheep only 22% of the total residues in fat is present as parent drug (Chiu & Lu, 1989).

Table 4.5. Total residue levels in liver, bile and fat of sheep dosed orally with ivermectin (300 ug/kg) or moxidectin (200 ug/kg)

Tissue	Drug	Days after dosing							Depletion half-life (d)
		1	3	5	7	14	21	28	
		(ppb)							
Liver	IVM	212	105	23	11	5	0.7	2	1.2
	MOX	135	n/a	n/a	50	n/a	n/a	17	
Bile	IVM				31	24	3	1	
	MOX	177	n/a	n/a	47	n/a	n/a	16	
Fat	IVM	245	153	63	32	24	13	10	1.8
	MOX	249	n/a	n/a	305	n/a	n/a	118	14 (27*)

n/a: Not available

Sources: Chiu & Lu, 1989; Chiu *et al*., 1990; Afzal *et al*., 1994

*Hayes, 1994.

Doramectin levels in liver, bile and fat of cattle determined up to 49 days after subcutaneous injection are given in Table 4.6. In cattle liver 14 days after treatment, doramectin levels are apparently higher than the total residue level for ivermectin or moxidectin (cf Table 4.4). The depletion half-life of doramectin in liver appears to be less than 7 days, somewhat longer than for total ivermectin and shorter than for total moxidectin residues; doramectin continues to be excreted in bile until at least 35 days after administration. Similarly to the other macrocyclic lactones, doramectin is lipophilic and residues in fat are detectable until 42 days after treatment in cattle; residue levels are higher than for ivermectin and lower than for moxidectin at the same time points. From the data in Table 4.6 depletion half-life of doramectin in fat is apparently around 7 days.

Table 4.6. Doramectin levels in liver, bile and fat of cattle dosed subcutaneously with the commercial formulation at 200 ug doramectin/kg

Tissue	Days after dosing					
	14	21	28	35	42	49
	(ppb)					
Liver	88	44	25	14 (16)	<10	<10
Bile	n/a	17	7	3	n/a	n/a
Fat	288	182	94	57 (17)	10	<10

Sources: Lynch & Logan, 1991; Magonigle & Lynch, 1992;
Figures in parenthesis at 35 and at 42 & 49 d: Hooke, Chick & Hennessy, 1993

4.1.3 Faeces

Differences in pharmacokinetic and metabolic behaviour are reflected in the differing faecal excretion profiles for ivermectin and moxidectin in cattle and sheep. The pattern of excretion in cattle faeces of total ivermectin residues following subcutaneous dosing indicates peak levels occur on day 2 (Table 4.7a). Maximum faecal levels of total moxidectin residues also occur during the second day after drug administration in cattle but at somewhat lower levels (Table 4.8) than for ivermectin. Excretion of moxidectin residues in faeces continues for more than 28 days at which time the cumulative excretion totals 58% of the dose, whereas cumulative excretion of ivermectin residues is 62% of the dose at 7 days. At the latter timepoint, 39-45% of ivermectin residues (Halley, Nessel & Lu, 1989) can be accounted for as parent drug compared with 22% of moxidectin residues at the same timepoint (Zulalian *et al.*, 1994). Other studies in cattle following subcutaneous dosing indicate that peak levels of ivermectin parent may occur at day 2 (Sommer & Steffanson, 1993), day 3 (Herd, Sams & Ashcroft, 1996), day 5 (Lumaret *et al.*, 1993), days 6 or 8 (Cook, Dadour & Ali, 1996) after treatment but ivermectin was generally not detectable in faeces after days 11-14, except by Herd *et al*. (1996) who recorded levels of 10 ppb after 28 days. These variations may be attributable to dietary differences between studies; Cook *et al*. (1996) showed that ivermectin concentrations in the faeces of pasture-fed cattle were lower throughout the 14 days post-injection period and peaked later than in grain-fed animals.

Percutaneous administration of ivermectin at 500 ug/kg resulted in higher initial concentrations in faeces (9 ppm dry matter) but by 5 days these were similar (2.8 ppm dry matter) to those following subcutaneous treatment (Sommer & Steffanson, 1993). Herd *et al*. (1996) observed peak ivermectin concentrations in faeces of 18.5 ppm dry matter 2 days after administering the pour-on formulation declining by 7 days to levels similar to those observed with the subcutaneous treatment. By contrast, topical treatment of cattle with moxidectin results in substantially lower faecal residue concentrations than after subcutaneous dosing and peak levels were not attained until 11 days (Table 4.8).

Table 4.7a. Total residue levels in faeces of cattle following ivermectin treatment (300 ug/kg) by subcutaneous injection

Days post treatment	1	2	3	4	5	6	7
Level (ppb)	292	806	597	513	301	333	273

Source: Chiu *et al*., 1990

Table 4.7b. Cumulative faecal excretion of total ivermectin residues in sheep following intraruminal administration of 200 ug/kg ivermectin

Days post treatment	0.5	1	1.5	2	3	4	5	6	7
Cumulative excretion (% dose)	0	13	39	60	80	90	92	95	96

Source: Ali, 1994

Table 4.8. Total residue levels in faeces (ppb) following moxidectin treatment by subcutaneous (S/c) and pour-on (Top) application to cattle and by oral drench to sheep

Days post treatment	Cattle		Sheep
	S/c (200 ug/kg)	Top (500 ug/kg)	Oral (200 ug/kg)
0	n/a	<2	n/a
1	n/a	6	3390
2	349	n/a	3030
3	267	14	1180
4	197	n/a	750
5	168	36	440
6	149	n/a	370
7	133	52	270
9	140	61	
11	101	211	
13	85	71	
14	n/a		
21	30		
28	40		

n/a: Not available
Source: Cyanamid Websters, 1996b.

Faecal excretion of total residues and parent drug in cattle for 14 days following subcutaneous injection of doramectin is shown in Table 4.9. These data indicate a broad peak level of excretion of total residues extending over the first 7 days after treatment; similarly the profile

of parent drug levels in faeces forms a broad-based peak over the same period, although a maximum concentration of 319 ppb doramectin occurred in faeces on day 3. Cumulative recovery in faeces was 87% of the dose by day 14 but doramectin levels still exceeded 100 ppb at this timepoint.

Table 4.9. Total residue and parent drug levels in faeces of cattle following subcutaneous injection of doramectin in commercial formulation at 200 ug/kg

Days post treatment	Total residues (ppb)	Doramectin (ppb)
0	268	124
1	543	227
2	400	232
3	549	319
4	519	277
5	562	263
6	561	294
7	515	171
8	443	234
9	397	247
10	347	256
11	255	196
12	269	216
13	223	127
14	239	133

Source: Magonigle & Lynch, 1992.

In sheep oral administration of moxidectin results in initial faecal concentrations 10 times higher than those observed in cattle after subcutaneous injection (Table 4.8) but by 7 days the levels in the two species are similar. At this stage cumulative excretion accounts for 43% of the dose and parent moxidectin comprises 25% of the total residues in sheep faeces (Afzal *et al.*, 1994). Faecal excretion of orally administered ivermectin by sheep is more rapid; by 7 days faecal residues account for 69% of the dose and 61-69% of these residues are present as parent drug (Halley *et al.*, 1989). Other studies have recorded >95% recovery of the total dose in faeces of sheep 7 days after intraruminal administration (Ali & Hennessy, 1996), two-thirds of this being recovered during the first 2 days (Table 4.7b).

4.2 Horses

Oral administration of the commercial formulation of ivermectin to horses results in an earlier and higher peak concentration in plasma than that observed in cattle or sheep (Table 4.1). Biological half-life is less than 3 days, similar to that in sheep. Measurements of faecal levels in horses are consistent with a rapid metabolism and excretion of the drug in this species; at 1 and 2 days after dosing faecal levels ranged from 1900 to 8500 ppb and 50 to 320 ppb, respectively and by 3 days faecal levels were below the detection limit of 50 ppb (Sams, 1993).

5. LABORATORY BIOASSAY OF TOXICITY OF MACROCYCLIC LACTONES

5.1 Dung Beetles

Laboratory investigations of the toxicity of macrocyclic lactones have critically examined effects on adult mortality, brood ball production and larval mortality in 10 species of dung beetles and results are summarised in Table 5.1. These studies have concentrated mainly on the effects of feeding on dung collected from cattle treated subcutaneously with ivermectin or abamectin; a similar study has been undertaken using dung of cattle treated with moxidectin and no comparable study has been reported with doramectin. The effect of dung from sheep treated orally with ivermectin has been examined in two studies and in one of these was compared with moxidectin. Effects of dung from horses are restricted to one study with a single species of dung beetle following moxidectin treatment.

5.1.1 Ivermectin/Abamectin

Studies of ivermectin and abamectin residue toxicity have used cattle faeces collected for up to 10 weeks after treatment and feeding periods for the adult dung beetles up to 60 days. In general, the mortality of sexually mature adults was unaffected although increased mortality and a slower rate of ovarian development were recorded in newly-emerged *O. binodis* feeding for an extended period of 8 weeks on dung of cattle treated with abamectin. However, it was concluded that under the normal 2-week period beetles feed on dung in the field abamectin given as a single injection to cattle has no permanent effects on ovarian development, mating or ovipositon of *O. binodis* (Houlding, Ridsdill-Smith & Bailey, 1991). Similarly, increased mortality of newly emerged *C. hispanus* and *O. belial* adults has been recorded after feeding for 14 days on dung containing ivermectin residues collected up to 8 days after treatment (Wardhaugh & Rodriguez-Menendez, 1988). *C. hispanus* displayed suppressed feeding activity and females had greatly reduced fat accumulation and distended guts with unusual contents after feeding for 43 days on dung collected after ivermectin treatment.

Ovarian development was completely suppressed in most females, and mating activity greatly reduced in *C. hispanus* feeding on dung collected up to 4 days after cattle were treated. Wardhaugh & Rodriguez-Menendez (1988) emphasise that prolonged exposures of 30 days or more to ivermectin residues at fixed levels used in their experiments are unlikely to occur under field conditions except for brood-caring species like *C. hispanus* which may store a single source of dung in burrows as a food source for prolonged periods of weeks or months. For the non brood-caring paracoprids, such as *B. bubalus* and *O. belial* which move from pad to pad, the shorter periods of exposure of 11-21 days to dung containing ivermectin residues were considered realistic in respect of feeding behaviour in the field.

Ivermectin residues in sheep dung increased the mortality of newly-emerged *E. fulvus* when feeding for 10 days on faeces voided during the first day after drenching but not subsequently (Wardhaugh *et al.*, 1993). It was deduced from the gut contents of survivors that feeding was inhibited and there was reduced fat accumulation and impaired ovarian development.

In sexually mature adult *O. gazella* and the closely related onthophagine *Diastellopalpus quinquedens* there are apparently no effects of ivermectin residues in cattle dung collected as early as 7 days after treatment on brood ball production. Similarly brood ball production by *Euoniticellus* spp. appears unaffected by ivermectin residues in cattle dung but is reduced in *E. fulvus* on sheep dung 1 day after oral treatment. There is evidence for an adverse effect of ivermectin residues on egg production by *O. binodis* when fed on dung collected during the first week after treatment of cattle.

Larval stages of all species examined are highly susceptible to ivermectin and abamectin residues in cattle and sheep dung; increased larval mortality, in many instances up to 100%, has been observed in dung collected during the first week after treatment. With *Onthophagus* spp. increased larval mortality has been observed in dung collected for up to 4 weeks after treatment (Table 5.1). Delayed larval development has been recorded in some studies, and in *O. gazella*, whilst morphology of the head capsules of dead larvae from day 2 dung indicated that toxicity occurred at the first instar stage, examination of surviving third-stage instars from day 7 dung indicated sub-lethal effects of ivermectin residues (Sommer & Overgaard-Nielsen, 1992). Similar measurements on the closely related *D. quinquedens* failed to find any differences in the head appendages of third-stage instar larvae collected from dung of treated and untreated animals (Sommer *et al.*, 1993b). These authors concluded that even closely related genera of dung beetles may vary substantially in their susceptibility to both lethal and sub-lethal effects of ivermectin. These observations illustrate the need for caution in extrapolating effects in one species to another.

5.1.2 Moxidectin

In the one study of the effects of moxidectin residues in cattle dung in which the experimental approach was similar to those described above on avermectin toxicity, no effects on adults and larvae of *O. gazella* and *E. intermedius* were recorded (Fincher & Wang, 1992). A further comparison of the larvicidal activity of moxidectin and abamectin was made by Doherty *et al.* (1994) in Australia using a laboratory assay in which serial dilutions of the injectable formulations of each compound were incorporated directly into dung collected from a housed steer fed lucerne pellets. Experimental pats were prepared containing a range of concentrations of either moxidectin or abamectin which doubled serially from 4 to 512 ppb wet weight of dung, covering the range of concentrations expected following subcutaneous injection of cattle at 200 ug/kg liveweight. Pairs of adult *O. gazella* were held on the experimental pats for 10-18 days and brood balls, adults, pupae and larvae collected 53 days after commencement. Neither moxidectin over the entire concentration range nor abamectin from 4 to 32 ppb dung reduced oviposition by *O. gazella*. No larvae survived at concentrations of 16 ppb abamectin or greater and survival was significantly reduced at 4 and 8 ppm. By contrast, larval survival on dung containing moxidectin was unaffected except at a concentration of 512 ppb when the percentage survival to adult was reduced to 7%, similar to the 5% survival observed on dung containing 8 ppm abamectin and compared with 72% survival on untreated control dung.

Moxidectin residues in sheep dung 2 days after oral drenching caused a 46% reduction in emergence of *Aphodius constans* larvae but had no adverse effect from 4 days after treatment (Cyanamid-Websters, 1996b). By comparison in the same study ivermectin reduced emergence of larvae of *A. constans* for at least 6 days after oral drenching.

Moxidectin residues in horse dung immediately following oral treatment have no apparent toxicological effect on feeding adults of *Anoplotrupes stercorosus* (Lumaret, 1996).

5.1.3 Doramectin

Using similar techniques to those described above, Clymer & Chappel (1993) added pure doramectin to faeces collected from cattle fed lucerne hay to give a range of concentrations from 0.25 to 250 ppb. Adult mating pairs of *O. gazella* were allowed to feed on experimental pats of the treated dung for 10 days and emerging beetles and brood balls collected between 30 and 50 days after commencement. A similar number of brood balls were produced at all concentrations of doramectin, indicating that this compound does not affect mating or oviposition of this particular species.

However, at the highest concentrations used (64 and 250 ppb) doramectin killed larvae of *O. gazella* at a very early stage of development and the data indicate a linear relationship between numbers of viable progeny and doramectin concentration in faeces. It was estimated that a level of 55 ppb reduced viable progeny by 90% (LC_{90}). However, Environment Australia considers that the LC90 is more likely to be in the order of 23 ppb based on statistical analysis of the raw data.

5.1.4 Milbemycin

There are apparently no data available on the toxicity of milbemycin against dung beetles or other insects.

5.2 Dung Flies

The toxicity of macrocyclic lactone residues to the development of eggs and larvae of dung breeding flies has been extensively examined under laboratory conditions with a particular emphasis on 'pest' species. Table 5.2 summarises the results of experiments with 7 species of dung flies using the faeces of cattle and sheep collected following treatment with ivermectin, abamectin or moxidectin. There are no reports available of similar assays of doramectin toxicity to dung flies.

Ivermectin residues in cattle faeces following subcutaneous treatment are highly toxic to the development of the larvae of the horn fly, *Haematobia irritans*, with mortalities of 100% initially and impaired development being evident in faeces collected for up to 8 weeks. Faeces of moxidectin-treated cattle exhibited lower toxicity against this species for a considerably shorter period of 3 days, with maximum mortality of approximately 75% on day 1 (Fincher, 1990). However, another study indicates that increased mortality may extend for at least 4 weeks after moxidectin treatment with a trend towards increasing mortality towards the end of this period, although not exceeding 77% at any stage (Miller *et al.*, 1994). In this latter study it was determined from spiking faeces with moxidectin that the LC_{90} against *H. irritans* was 77.5 ppb. In similar experiments Doherty *et al.* (1994) examined the toxicity of cattle faeces spiked with various concentrations of the commercial formulations of moxidectin or abamectin against the closely related buffalo fly, *Haematobia irritans exigua*. Whereas abamectin levels of 4 ppb or greater caused 100% mortality there were no toxic effects of moxidectin until levels exceeded 64 ppb. Although the results of this experiment could be confounded by differences in the excipients used in the two commercial formulations this seems unlikely to change the overall conclusion that moxidectin is intrinsically less toxic than abamectin to the buffalo fly.

Residues of ivermectin in cattle faeces show similar toxic effects on the development of eggs of several *Musca* spp. Duration of effect varies between species and between experiments but the data in Table 5.2 indicate that, when given subcutaneously to cattle, ivermectin reduces larval development of *Musca* spp. in dung for at least 2 weeks and up to 7 weeks after treatment. Of particular interest in the Australian context is the impact on the bushfly *M. vetustissima* which exhibits reduced larval development for at least 4-5 weeks when eggs are laid in dung of cattle treated with ivermectin or abamectin. Toxic effects of ivermectin residues against larvae of the house fly, *M. domestica*, have been recorded for up to 20 days after treatment of cattle. By contrast there was no effect on development of *M. domestica* and *M. vetustissima* larvae on dung collected as early as 3 days after subcutaneous treatment of cattle with moxidectin. Faecal excretion of ivermectin residues by sheep following oral treatment also prevents development of *M. vetustissima* but for a considerably shorter period of 6-7 days compared with subcutaneous treatment of cattle. Effects of ivermectin on *N. cornicina*, an obligate dung feeder, under European conditions, show similar differences between cattle and sheep faecal residues, with larval development being reduced for up to 32 days and 7 days, respectively. Again, residues in sheep faeces following oral moxidectin treatment were toxic to *N. cornicina* development for a shorter period than following oral ivermectin treatment.

In Australia, there has been considerable interest in the influence of ivermectin residues in sheep dung on the breeding behaviour of the sheep blowfly, *Lucilia cuprina*. Adult females exhibited impaired ovarian development, reduced fecundity and reduced survival when fed continuously on sheep dung collected within 24 hours of oral treatment with ivermectin (Mahon & Wardhaugh, 1991; Mahon *et al.*, 1993; Cook, 1991). Although these effects have been recorded on sheep dung collected up to 138 hours after ivermectin treatment (Mahon & Wardhaugh, 1991), in other studies such effects have been confined to the first 24 hours (Mahon *et al.*, 1993). Variation in toxicity may be attributable to differences in feed intake and pasture quality and the consequent effect on protein content of the faeces which is known to influence ovarian development in *L. cuprina* (Mahon *et al.*, 1993). Adult males showed aberrant mating behaviour when feeding on sheep dung collected over the 6 days following an ivermectin drench (Cook, 1993).

Table 5.1. Summary of laboratory investigations to determine the toxicity to dung beetles of faeces of cattle, sheep and horses following treatment with ivermectin (Ivm), abamectin (Abm) and moxidectin (Mox)

Species	Compound and route	Source of faeces	Collection times	Feeding period (adults)	Adult Mortality	Brood ball production	Larval mortality	Reference
Onthophagus gazella	Ivm, S/c	Cattle	Wkly for 10 wks	7 d	No effect	No effect	100% wk 1, 93% wk 2. No effect wks 3-10	Fincher, 1992
-do-	Ivm, Abm, S/c	Cattle	Up to 35 d	(not stated)	No effect	(Not stated)	Ivm: 100% to 10 d Abm: 100% to 21 d	Picton & Burrows, cited by Roncalli, 1989
-do-	Ivm, S/c	Cattle	2, 7, 17 d	12 d	(Not stated)	No effect	~ 100% up to 7 d	Sommer & Overgaard Nielsen, 1992
Onthophagus binodis	Abm, S/c	Cattle	1, 2, 4, 8, 11 wks	16 d	No effect	67% reduction wk 1	100% wk 1, increased until wk 4. No effect wks 8 & 11	Ridsdill-Smith, 1988
-do-	Abm, S/c	Cattle	3, 4, 5 d	2 & 8 wks	30-40% increase in newly emerged	Reduced egg production on 8 wk feeding	(Not stated)	Houlding et al., 1991
Diastellopalpus quinquedens	Ivm, S/c	Cattle	2, 8, 16 d	5 d	(Not recorded)	No effect	72% in 2 d 10% in 8 d 6% in 16 d	Sommer et al., 1993a
Euoniticellus intermedius	Ivm, S/c	Cattle	Wkly for 10 wks	7 d	No effect	No effect	100% wk 1 No effect wks 2-10	Fincher, 1992

Species	Compound and route	Source of faeces	Collection times	Feeding period (adults)	Adult Mortality	Brood ball production	Larval mortality	Reference
Euoniticellus fulvus	Ivm, S/c	Cattle	1 & 10 d	29 d	No effect	No effect in 10 d	100% in 1 d Delayed development in 10 d	Lumaret et al., 1993
Copris hispanus	Ivm intramusc.	Cattle	1, 2, 3, 4, 8, 16, 32, 64 d	60 d	No effect in matures, High in newly emerged	Reduced in 3 d	100% on 3 & 8 d	Wardhaugh & Rodriguez-Menendez, 1988
Bubas bubalus	-do-	Cattle	1-32 d	32 d	No effect	(Not recorded)		-do-
Onitis belial	-do-	Cattle	1-32 d	32 d	Increased in newly emerged	(Not stated)	(Not stated)	-do-
Onthophagus gazella *E. intermedius*	Mox, S/c	Cattle	1-42 d “	7 d	No effect	No effect	No effect	Fincher & Wang, 1992
E. fulvus	Ivm oral	Sheep	1, 2, 5, 10, 16, 32 d	21 d	No effect on matures, Increase on 1 d in newly emerged	Reduced on 1 d	100% on 1 & 2 d	Wardhaugh et al., 1993
Aphodius constans	Mox, oral	Sheep	2-38 d	(Not stated)	(Not stated)	(Not stated)	46% on 2 d, No effect after 4 d	Cyanamid Websters, 1996b
	Ivm, oral	“	“	“	“	“	99-100% until 5 d, No effect after 10 d	-do-

Species	Compound and route	Source of faeces	Collection times	Feeding period (adults)	Adult Mortality	Brood ball production	Larval mortality	Reference
Anoplotrupes stercorosus	Mox, oral	Horse	1-35 d	(Not stated)	No effect	(Not stated)	(Not stated)	Lumaret, 1996
	Ivm, oral	"	"		"	"	"	"

*S/c: subcutaneous injection

Table 5.2. Summary of laboratory investigations of the toxicity of ivermectin (Ivm), abamectin (Abm) and moxidectin (Mox) by subcutaneous (S/c) or intramuscular (i/m) route in cattle and sheep dung to the development of Diptera larvae

Species	Compound and route	Dung source	Collection times	Period of reduced larval development	Reference
Haematobia irritans (Horn fly)	Ivm, S/c	Cattle	1-49 d	35 d	Schmidt, 1983
	"	"	7-70 d	56 d	Fincher, 1992
	"	"	1-28 d	28 d	Miller *et al.*, 1994
	"	"	1-42 d	14 d	Sommer *et al.*, 1992
	Mox, S/c	"	1-28 d	3 d	Fincher, 1990
Neomyia cornicina	Ivm, i/m	"	1-32 d	32 d	Wardhaugh & Rodriguez-Menendez, 1988
	Ivm, S/c	"	1-42 d	14 d	Sommer *et al.*, 1992
	"	"	1-10 d	10 d	Lumaret *et al.*, 1993
	Ivm, oral	Sheep	2-38 d	7 d	Cyanamid-Websters, 1996b
	Mox, oral	"	"	2 d	
Musca autumnalis (face fly)	Ivm, S/c	Cattle	1-33 d	46 d	Madsen *et al.*, 1990
	"	"	1-42 d	14 d	Sommer *et al.*, 1992
Musca domestica (house fly)	"	"	1-33 d	20 d	Madsen *et al.*, 1990
	"	"	3-7 d	7 d	Wardhaugh *et al.*, 1996
	Mox, S/c	"	"	No effect	"
Musca vetustissima (bushfly)	Abm, S/c	"	7-77 d	28 d	Ridsdill-Smith, 1988
	Abm, S/c	"	3-35 d	35 d	Wardhaugh & Mahon, 1991
	Ivm, S/c	"	3-35 d	28 d	Wardhaugh *et al.*, 1996
	Mox, S/c	"	3-35 d	No effect	"
	Ivm, oral	Sheep	1-28 d	6 d	Wardhaugh & Mahon, 1991
	"	"	1-32 d	7 d	Wardhaugh *et al.*, 1993
Stomoxys calcitrans (stable fly)	Ivm, S/c	Cattle	1-49 d	7 d	Schmidt, 1983
Musca nevelli	Ivm, S/c	"	1-56 d	49 d	Kruger & Scholtz, 1995

6. FIELD EVALUATION OF DUNG COLONISATION AND TOXICITY

6.1 Cattle

The pioneering field study of Wall & Strong (1987), which drew attention to the potentially serious ecological consequences of long-term ivermectin treatment on the colonisation of cattle dung pats on pasture by Coleoptera and Diptera, has generated many investigations on this topic over the past decade. The results of 12 field studies of the impact of ivermectin or abamectin treatment given subcutaneously, topically or by an intraruminal sustained release bolus on the insect fauna of cattle dung are summarised in Table 6.1. In one of these studies moxidectin treatment was also examined. Most studies have been undertaken under temperate conditions in the northern hemisphere where the climate and species diversity are quite different to those under Australian field conditions but includes the genus *Aphodius* which is prevalent in Australia. Work in South Africa and Spain has provided information of particular relevance to the *Scarabaeidae* tribes which are represented in the introduced dung beetle fauna of Australia. By comparison, the influence of macrocyclic lactone residues on colonisation of cattle dung pats and associated toxic effects on dung beetles has received limited attention under field conditions in Australia, there being only one such study reported (Wardhaugh & Mahon, 1991).

It is evident from the results of the European studies that variable responses have been observed in the Coleoptera and Diptera populations colonising the dung of cattle treated subcutaneously or topically with ivermectin or abamectin. Some of this variation may be attributable to differences in the period of observation after natural or artificial deposition of the dung pat on pasture. However, some general conclusions are possible from the combination of short- and long-term observations extending up to 105 days after dung deposition. Numbers of adult Coleoptera were generally not affected by ivermectin or abamectin residues in dung collected as early as 1 day after subcutaneous or topical treatment; on the other hand, larval numbers were generally reduced in dung deposited up to 17 days after treatment. Reduced numbers of dipteran larvae were also recorded in dung deposited up to 30 days after subcutaneous ivermectin treatment, but differential effects were noted between the sub-orders of Diptera. Nematocera larvae were not affected in several studies whereas reduced numbers were recorded in pats collected up to 10 days after treatment in one Danish study; larvae of Brachycera and Cyclorrhapha were apparently more sensitive to dung residues than Nematocera.

Although outside the terms of reference of this review, it is of interest to note that studies have been made of dung pat colonisation under European conditions during the period of continuous release of ivermectin from an intraruminal bolus designed to treat at 40 µg/kg/day for 120 days (Table 6.1). Observation periods on artificial and naturally voided pats ranged up to 100 days after deposition on pasture.

Generally the effects were similar to those observed in dung collected during the first 2-3 weeks following a single subcutaneous or topical dose of ivermectin/abamectin. Larval numbers of Coleoptera were reduced or totally absent and there was evidence of impaired larval development in *Scarabaeidae*. Similar effects were observed on the number of Diptera larvae although Cyclorrhapha were more adversely affected than Nematocera.

In contrast to the effects of ivermectin and abamectin treatment, pats prepared from faeces collected up to 21 days after subcutaneous dosing of cattle with moxidectin did not affect larval numbers of Coleoptera or Diptera species colonising the dung (Strong & Wall, 1994).

Studies in Spain (Lumaret *et al*., 1993) and Australia (Wardhaugh & Mahon, 1991) have given results that contrast with the Northern European observations of the impact of ivermectin and abamectin on adult dung beetles; increased numbers of adult Coleoptera were recovered in pats prepared from faeces deposited up to 17 days after subcutaneous treatment, which suggests that dung containing avermectin residues may be more attractive to the particular *Scarabaeid* species of dung beetles present in these environments. Conversely, in South Africa a reduction in the diversity and abundance of dung beetles colonising pats was observed in faeces deposited 1 month after cattle were treated with ivermectin (Scholtz & Kruger, 1995). However, consistent with the longer term European studies, no changes could be detected in the adult population recovered from faeces deposited 2 and 3 months after treatment.

The attractiveness to dung beetles of faeces of cattle treated with ivermectin/abamectin has been examined using dung-baited pitfall traps under a range of environments in Australia, Spain, Denmark and Africa. In trials undertaken at two locations in south-eastern Australia, Wardhaugh & Mahon (1991) used dung collected from cattle prior to treatment and 3, 25 and 35 days after injection subcutaneously with abamectin (200 µg/kg). These collections were used to prepare dung-baited pitfall traps to monitor arrival rates of dung beetles. Five species of dung beetle were caught, the 3 native species *O. australis*, *O. pexatus*, which were generally most abundant, and *O. pentacanthus*, present in small numbers, and the introduced species *E. fulvus* and *O. taurus*. Dung collected 3 days after treatment with abamectin attracted more beetles than dung from untreated animals and this effect was still evident with day 25 but not day 35 dung. Wardhaugh & Mahon (1991) were unable to explain the enhanced attractiveness of dung from avermectin-treated animals, considering the possible presence of a volatile metabolite of avermectin or, more likely, changes in gut flora stimulated by the therapy.

Similar experiments by Lumaret *et al.* (1993) in Spain using pitfall traps baited with dung collected from cattle 2 to 31 days after subcutaneous treatment with ivermectin showed that dung collected on days 7, 10 and 17 attracted more adult beetles than that from untreated animals. Since day 7 and 10 baits contained ivermectin but at day 17 the concentration was below the detection limit, Lumaret *et al.* (1993) agreed with the suggestion of Wardhaugh & Mahon (1991) that changes in faecal composition, elicited by modification of gut flora following ivermectin therapy, may have been responsible for its increased attractiveness. Although differential effects on individual species were not recorded, it is noteworthy that in addition to several *Aphodius* spp. the species collected included *O. taurus* and *E. fulvus*, both of which are important introduced species in Australia.

The attractiveness of dung from ivermectin treated cattle to afrotropical Scarabaeid dung beetles has also been assessed under field conditions in Tanzania and Zimbabwe (Holter, Sommer & Gronvold, 1993a; Holter *et al.* 1993b). In these experiments beetles were caught in pitfall traps baited with dung collected at intervals from 2 to 21 days after subcutaneous injection of cattle with ivermectin (200 ug/kg). In three Tanzanian trials the species trapped were predominantly of the Scarabaeid tribe Onthophagini and there was an overall tendency for the beetles to be more attracted to the dung of untreated cattle. However, although the number caught was small, there was evidence of a consistent preference by *E. intermedius* for the dung of ivermectin treated animals. At three locations in Zimbabwe, 4 species of Scarabaeid dung beetles were captured in the pitfall traps, *D. quinquedens, L. militaris, O. viridulus* and *E. intermedius*. In traps containing dung collected 2 and 8 days after ivermectin treatment there were significantly more of the Oniticellini, *L. militaris* and *E. intermedius*, than with day 16 or control dung, whereas preferences for treated and untreated dung were similar for the other species.

In Denmark, Holter *et al.* (1993a, b) used pitfall traps baited with the dung of ivermectin treated cattle collected 3 to 30 days after treatment and in some instances dung of untreated animals to which ivermectin was added. In one experiment the Scarabaeid, *Aphodius*, and the Hydrophilids, *Sphaeridium* and *Cercyon* preferred control dung from untreated cattle, but in two further experiments there was no significant difference in attractiveness for any species between the two dung types.

From these inconsistent responses between the Danish, Tanzanian and Zimbabwe trials and the observations in Australia by Wardhaugh & Mahon (1991), Holter *et al.* (1993a) speculated that differences in attractiveness of dung may be associated with the taxonomic position of the beetles and it was tentatively suggested that there may be a preference for dung from ivermectin-treated cattle within the tribe Oniticellini.

These observations illustrate the complexity of interpreting changes in the abundance of dung beetle fauna associated with avermectin treatment. It cannot necessarily be assumed that an impoverished fauna in dung containing avermectin residues is due to mortality rather than a behavioural response since it is clear that some species of colonising beetles sometimes prefer dung from untreated cattle and may avoid pats from treated animals. However, since avermectin therapy can also lead to increased attractiveness of the dung to some afro-tropical species of beetles the adverse effects of avermectins on reproduction, larval development and survival may be accentuated in terms of overall outcomes on the population dynamics of these particular species.

6.2 Sheep

There is only one reported study in Australia using pitfall traps baited with dung of sheep treated orally with ivermectin 18, 42 and 138 hours previously (Wardhaugh & Mahon, 1991). Again enhanced attractiveness to the more abundant species *O. australis* was evident but only with dung collected at 18 hours and no effect could be detected on the other species present, *O. posticus.*

6.3 Horses

No studies have been reported on the influence of macrocyclic lactone residues in horse dung on colonisation by dung beetles.

Web users: the following landscape pages may not print successfully. They are available at http://www.affa.gov.au/nra/chemrev.html - select the attachment to the Macrocyclic lactomes report.

Table 6.1. Summary of investigations to determine the effect of ivermectin (Ivm), abamectin (Abm) and Moxidectin given subcutaneously (S/c), topically or by sustained release bolus to cattle on the colonisation of dung pats in the field by Coleoptera and Diptera

Country	Method of pat formation	Treatment	Collection times after treatment	Observation Period	Species and abundance	Effect of treatment	Reference
IVERMECTIN / ABAMECTIN							
England	Artificial	Bolus (Ivm, 40 ✪g/kg/d)	During 6 d	20-100 d	**Coleoptera:** *Scarabaeidae* 89.4% - mainly *Aphodius spp*. *Geotrupidae* 4.7% *Staphylinidae* 3.1% *Carabidae* 1.3% *Hydrophilidae, Elateridae* **Diptera**: *Bibionidae* 56.6% *Stratiomyidae* 18.6% *Chironimidae* 11.8% *Mycetophilidae, Muscidae, Sepsidae, Syrphidae*	Adults, larvae and pupae - almost total absence for 100 d Larvae - almost totally absent	Wall & Strong, 1987
England	Artificial	S/c (Ivm)	2, 7, 14, 21d	7 - 42 d	**Coleoptera**: *Scarabaeidae* - mainly *Aphodius spp*. *Hydrophilidae,Carabidae, Staphylinidae* **Diptera**: *Anisopodidae, Anthomyiidae, Bibionidae, Fannidae, Muscidae, Psychodidae, Stratiomyidae, Sepsidae, Tipulidae*	Adults - no significant effect Larval development reduced in 2 & 7 d pats Cyclorrhapha - absent in 2, 7 & 14 d , reduced in 21 d pats. Nematocera - no effect.	Strong & Wall, 1994

Country	Method of pat formation	Treatment	Collection times after treatment	Observation Period	Species and abundance	Effect of treatment	Reference
England	Artificial	S/c (Abm)	3, 17, 35 d	3 - 105 d	**Coleoptera**: *Staphylinidae - Aleocharinae, Oxytelinae, Xantholininae, Philonthus spp.* *Hydrophilidae* - Cercyon, Sphaeridium, Cryptopleureum spp.* *Scarabaeidae*- Aphodius spp.* *Ptiliidae, Histeridae* **Diptera**: *Psychodidae*, Ceratopogonidae, Scatopsidae, Stratiomyidae, Sepsidae*, Sphaeroceridae*, Anthomyiidae*,Fannidae, Muscidae**	Adults - no effect *Larvae- reduced in 3 and 17d pats up to 28 days after placement *Reduced live larvae and pupae in 3 and 17 d pats through to 28 days after placement	Baggott *et al*., 1994
England	Artificial	Bolus (Ivm, 43 ✪g/kg/d)	21 d	7 - 42 d	**Coleoptera**: *Scarabaeidae* *Hydrophilidae* *Staphylinidae* **Diptera**: Cyclorrhapha: Nematocera:	*Scarabaeidae* - reduced egg and larval numbers, no effect on adults, impaired larval development *Hydrophilidae* - larvae absent -No larvae present -No significant effects.	Strong *et al*., 1996

Country	Method of pat formation	Treatment	Collection times after treatment	Observation Period	Species and abundance	Effect of treatment	Reference
Germany	Artificial	S/c (Ivm)	2 d, then weekly for 16 weeks	2 - 7 d	**Coleoptera**: *Staphylinidae* (42 spp.) *Hydrophilidae* - *Sphaeridum* (3 spp.) *Cercyon spp.* *Scarabaeidae* (8 spp.) *Histeridae* **Diptera**: Brachycera: *Sepsidae, Muscidae, Sphaeroceridae, Stratiomyidae* Nematocera: *Phryneidae, Psychodidae, Chironomidae, Lycoriidae*	No effect on adults or larval development Significant reduction of Muscidae and Sepsidae larvae Not affected	Schaper & Liebisch, 1991
Germany	Naturally voided on pasture	Bolus (Ivm, 58-71 ✪g/kg/1d)	During 120 d treatment	3, 7, 14, 28 d	**Coleoptera**: *Scarabaeidae* - mainly *Aphodius spp*. (10) *Hydrophilidae* - *Cercyon spp*. (7) *Sphaeridium spp*. (2) *Megasternum spp*. (1) *Cryptopleureum spp*. (3) *Staphylinidae* - (28 spp.) **Diptera**	No effect on adult numbers or frequency Larvae - numbers reduced Reduced larval numbers	Barth *et al*., 1993
Germany	Naturally voided	S/c (Ivm)	3, 7, 14, 28d	3-63 d	**Coleoptera**: *Scarabaeidae* 4.1% - 10 spp. mainly *Aphodius* *Hydrophilidae* 27.2% - 14 spp.	Adults - no effect on total numbers or individual spp. Larvae - reduced in 7 and 14 d	Barth *et al*., 1994

Country	Method of pat formation	Treatment	Collection times after treatment	Observation Period	Species and abundance	Effect of treatment	Reference
					mainly *Cercyon* *Staphylinidae* 68.7% - 28 spp. *Histeridae, Dryopidae, Ptilidae, Curculionidae* (low numbers) **Diptera**: (not speciated)	pats up to 21 days after deposition; higher in 21-63 day old pats; no effect 3-63 day pats overall Larvae reduced in - 3 d & 7 d pats for 63 days, 14 d pats for 35 days, 28 d pats for 21 days after deposition	
Spain	Artificial	S/c (Ivm)	2, 4, 7, 10, 17, 24, 31 d	1 - 23 d	**Coleoptera**: 90% of total numbers: *Onthophagus taurus,O. furcatus, Caccobius schrebei,Euoniticellus fulvus, Aphodius spp.* (4) **Diptera**: (not speciated)	Adults - higher numbers in 7, 10, 17 d pats Larvae reduced in 2-10 d pats	Lumaret *et al*., 1993
Denmark	Artificial	S/c (Ivm)	1, 10, 20, 30d in 2 years	3 - 86 d	**Coleoptera**: *Staphylinidae* 60-80% *Scarabaeidae- Aphodius spp.* *Hydrophilidae - Cercyon spp., Sphaeridium spp.* **Diptera**: Nematocera: *Ceratopogonidae, Chironomidae, Sciaridae, Psychodidae* Cyclorrhapha: *Sphaeroceridae, Sepsidae, Muscidae*	Larvae of *Aphodius* reduced in 1 d pats - Reduced larvae in 1 and 10 d pats; reduced pupae in 1 d pats - Reduced larvae and pupae in 1, 10, 20 and 30 d pats	Madsen *et al*., 1990

Country	Method of pat formation	Treatment	Collection times after treatment	Observation Period	Species and abundance	Effect of treatment	Reference
Denmark	Artificial	S/c, Topical (Ivm)	1/2, 13/14, 28/29, 42/43 d	3 - 45 d	**Coleoptera**: *Aphodius spp.* **Diptera**: Nematocera: *Ceratopogonidae* 79%, *Psychodidae* 12%, *Chironomidae* 6%, *Anisopodidae* 2% Cyclorrhapha:	Reduced larvae in 1/2 d pats No effect - Reduced larvae in 1/2 & 13/14 d pats after Topical - Reduced larvae in 1/2, 13/14 and 28/29 d pats after S/c	Sommer *et al.*, 1992
S. Africa	Artificial	S/c (Ivm)	1, 2, 3 mths	1 d	**Coleoptera**: *Scarabaeidae* (49 spp.) *Aphodiinae* (13 spp.) *Staphylinidae* (10 spp.) *Hydrophilidae* (2 spp.)	Diversity and rank/ abundance decreased at 1mth. Returned to control levels at 2 and 3 months	Scholtz & Kruger, 1995
Australia	Artificial	S/c (Abm)	3 d	2, 4, 8 d	**Coleoptera:** Predominantly *Onthophagus australis*	Increased number of beetles and tunnels at 4 and 8 d	Wardhaugh & Mahon, 1991
MOXIDECTIN							
England	Artificial	S/c	2, 7, 14, 21 d	7 - 42 d	**Coleoptera**: *Scarabaeidae* - mainly *Aphodius spp.* *Hydrophilidae,Carabidae, Staphylinidae* **Diptera**:	No significant effects	Strong & Wall, 1994

Country	Method of pat formation	Treatment	Collection times after treatment	Observation Period	Species and abundance	Effect of treatment	Reference
					Anisopodidae, Anthomyiidae, Bibionidae, Fannidae, Muscidae, Psychodidae, Stratiomyidae, Sepsidae, Tipulidae		

7. INFLUENCE OF MACROCYCLIC LACTONES ON DUNG DEGRADATION

7.1 Cattle

The original association between absence of dung-degrading insects, particularly beetles, in the faeces of calves treated with an ivermectin bolus and the failure of dung pats to degrade at a normal rate (Wall & Strong, 1987) has stimulated much research on the influence of macrocyclic lactones on pat decomposition. Table 7.1 summarises the results of 13 subsequent experiments which examined ivermectin or abamectin and one experiment which examined doramectin effects on the degradation of artificial or naturally voided cattle dung pats. No experiments have been reported on the degradation of dung from cattle treated with moxidectin. Most of the data with ivermectin and abamectin have been obtained under European field conditions and have included application subcutaneously, topically and by intraruminal sustained release bolus but consistent results have not been recorded between countries.

Three studies conducted under Danish field conditions (Madsen *et al*., 1988, 1990; Sommer *et al*., 1992) reported slower rates of degradation of dung collected from cattle within the first 3 weeks of treatment with ivermectin subcutaneously or topically. However, other experiments in Germany, France, England and Scotland (see Table 7.1) were generally unable to detect any significant change in degradation rate or in the long-term accumulation of dung on the paddock following subcutaneous, topical or bolus treatment with ivermectin. In one experiment there was no long-term effect of abamectin given subcutaneously on degradation of dung collected 3 days after treatment despite an initial reduction in the rate of disappearance.

Differences between the Danish and other European data may reflect differing species diversity in the dung fauna of the various countries. In Denmark, Dipteran larvae constituted more than 80% and adult dung beetles no more than 10% of the invertebrate population of dung pats (Madsen *et al.*, 1990; Sommer *et al.*, 1992). The activity of larvae of dung flies plays a dominant role in disrupting the integrity of the dung pat during the first week or so after deposition under Danish conditions. The high sensitivity of many Diptera, including the important yellow dung fly, *Scatophaga stercoraria* (Strong & James, 1993), may therefore explain the negative effect of ivermectin residues on dung degradation in Denmark. In the German field trials it was noted that the insect community comprised 50% Diptera larvae, 30% Coleoptera larvae and 20% adult beetles (Barth *et al.*, 1994). In the United Kingdom, beetles can be the most abundant of the insect fauna colonising the dung pat (see Wall & Strong, 1987) or there may be similar numbers of both Coleoptera and Diptera larvae present (see Baggott *et al.*, 1994). In the latter study an initial reduced degradation of dung collected 3 days after treatment of cattle with abamectin was associated with survival rates of 11% and 23% for Coleoptera and Diptera larvae, respectively, in the treated compared to control pats.

Under field conditions in Zimbabwe freshly deposited dung is rapidly buried by onthophagine and oniticelline dung beetles and may largely disappear within 24 hours (Sommer *et al.*, 1993a). The lack of effect on dung degradation in this study is therefore consistent with the

absence of effect on adult beetle mortality of ivermectin residues in dung. Similarly, in Australia the increased rate of burial of cattle dung collected shortly after abamectin treatment was consistent with the observed increases in adult beetle populations and their tunnelling activity.

The study which showed no influence of doramectin residues on cattle dung degradation was undertaken in the upper mid west of the USA where, under cool, moist conditions, dung feeding Coleoptera larvae, predominantly of the sub family *Aphodiinae*, constituted the bulk of the invertebrate biomass collected 28 days after pat deposition (Moon, 1992). Unfortunately, in this particular experiment dung collected 4 days after doramectin treatment was placed on pasture when the prevailing conditions were hot and dry and colonisation by invertebrates was extremely sparse. As acknowledged in the study report, absence of an effect of doramectin on the disappearance of dung collected shortly after treatment should therefore be interpreted with caution and further examination of the potential effects of doramectin is necessary.

7.2 Sheep

Only one investigation has been reported of the effects of macrocyclic lactone treatment of sheep on the degradation of dung deposited on pasture under Australian conditions. In this study (Rugg, 1995) the accumulation of dung was measured on paddocks which grazed sheep during and for 140 days following treatment with ivermectin released from an intraruminal capsule at 1.6 mg/day for 100 days. No significant differences were found at any sampling time throughout the 240 days in the dry weight of dung collected at multiple sites on each paddock between the ivermectin treatment, albendazole treatment at 32.5 mg/day for 100 days using a similar capsule or the untreated control group.

7.3 Horses

Investigations in the USA of the impact of ivermectin treatment of horses on dung degradation on pasture have produced conflicting results. Herd, Stinner & Purrington (1993) measured dry weights serially over about 3 months after placing artificial faecal deposits, or copromes, on pasture using dung collected 3 days after treatment of horses with ivermectin. Copromes from ivermectin treated animals placed on paddocks in June disintegrated more slowly than those from untreated controls. However, there were no significant differences between the two treatments in the rate of disappearance of copromes placed out in August. In another experiment Herd *et al*. (1993) measured the dispersal of naturally voided horse dung on ungrazed pasture over a 9-month period by measuring changes in circumference of pats and visual assessment of dispersal. Compared with the dung pats of untreated and oxibendazole-treated controls there was a substantially slower reduction in the surface area of those originating from horses over the 7 days following ivermectin treatment.

No studies have been reported under Australian conditions of the influence of macrocyclic lactone treatment on the degradation of horse dung.

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Table 7.1. Summary of investigations to determine the effect of ivermectin (Ivm), abamectin (Abm) and doramectin given subcutaneously (S/c), topically or by sustained release bolus to cattle on the degradation rate of dung

Country	Method of pat formation and location	Treatment	Collection times after treatment	Observation period	Degradation effect	Reference
IVERMECTIN / ABAMECTIN						
Denmark	Artificial in pots	S/c (Ivm)	1 d	98 d	Delayed by up to 53 d	Madsen *et al.*, 1988
	Artificial in field	S/c (Ivm)	1, 10, 20, 30 d	86 d	Delayed for 1-20 d dung	Madsen *et al.*, 1990
	Artificial in field	S/c, Topical (Ivm)	1/2, 13/14, 28/29, 42/43 d	45 d	Delayed for 1/2 d dung Delayed for 1/2 & 13/14 d dung	Sommer *et al.*, 1992
Germany	Naturally voided on pasture	120 d bolus (Ivm)	During treatment	259 d	Slight delay in reduction of pat area (not significant)	Barth *et al.*, 1993
	Artificial in field	S/c (Ivm)	2 d then weekly for 16 weeks	140 d	None	Schaper & Liebisch, 1991
	Naturally voided on pasture	S/c (Ivm)	3, 7, 14 & 28 d	63 d	None	Barth *et al.*, 1994
Scotland	Naturally voided on pasture	Topical (Ivm)	7 d	63 d	None	McKeand *et al.*, 1988

Country	Method of pat formation and location	Treatment	Collection times after treatment	Observation period	Degradation effect	Reference
Zimbabwe	Artificial in field	S/c (Ivm)	2, 8, 16 d	5 d	None	Sommer *et al.*, 1993a
Australia	Artificial in field	S/c (Abm)	3 d	42 d	Increased	Wardhaugh & Mahon, 1991
England	Artificial in field	Bolus	During (6 d)	100 d	Decreased	Wall & Strong, 1987
	Artificial in field	S/c (Abm.)	3, 7, 35 d	168 d	Reduced in 3 d dung for up to 126d. No effect at 168d	Baggott *et al.*, 1994
	Naturally voided on pasture	S/c, Bolus (90 or 120 d)(Ivm)	5, 35, 63 d During and up to 30 d after	63-98 d in 2 grazing seasons	None None	Wratten *et al.*, 1993
	Natural accumulation on paddock (total)	S/c, bolus (Ivm)	During entire grazing period (7-8 mths)	7-8 mths in two successive grazing seasons	None	Wratten *et al.*, 1993
France	Naturally voided on pasture	S/c, Top (Ivm)	21, 28 d	28 d	None	Robin, 1988
	-do-	-do-	3, 7, 14, 21, 28 d	56 d	None	Robin, 1991

Country	Method of pat formation and location	Treatment	Collection times after treatment	Observation period	Degradation effect	Reference
DORAMECTIN						
USA	Naturally voided on pasture	S/c	4, 32, 60 d	16 months over 2 grazing seasons	None	Moon, 1992.
	Artificial on pasture	S/c	4, 32, 60 d	28, 112 & 280 d	None	Moon 1992

8. USE PATTERNS OF MACROCYCLIC LACTONES IN AUSTRALIA AND RISK EXPOSURE OF DUNG BEETLE POPULATIONS

8.1 Cattle

The epidemiology of infection and the economic importance of different species of gastrointestinal parasites are delineated by two distinct climatic zones, the temperate and the tropical/subtropical, in each of which the prevalence of parasitism in cattle is largely determined by rainfall. The temperate zone can be further subdivided on the basis of summer or winter rainfall pattern. Throughout the temperate zone *Ostertagia ostertagi*, *Trichostrongylus axei* and *Cooperia oncophora* are the main species contributing to parasitic gastroenteritis in cattle, but *O. ostertagi* is the most important of the three and recommended treatment programs are principally directed at preventive control of this parasite in young cattle. It is generally accepted that only young cattle up to 2 years of age need to be treated on a regular basis and both macrocyclic lactone and benzimidazole carbamate anthelmintics are effective and recommended for this purpose (see Hall, 1990). Throughout the southern, or winter rainfall, region of the temperate zone where autumn calving is practised, a drench at weaning in December to January is recommended with follow-up treatments in March-April, and, on a minority of properties, in July-August (Anderson, Donald & Waller, 1983). Further treatment the following January of 2-year-old weaners is designed to prevent Type II ostertagiasis caused by the emergence of inhibited larvae which accumulate over the late spring and summer. In southern NSW where spring calving is preferred, a weaning drench is recommended in March-April with follow-up treatment in May-June, and again, on a minority of properties, in July-August (Hall, 1990). On the Northern Tablelands of New South Wales in the summer rainfall zone, a single drench at weaning provides adequate control with little or no production response to follow-up treatments (Anderson *et al*., 1983). Since cattle are usually immune to *Ostertagia* by 2 years of age routine use of anthelmintics should not be necessary in mature animals except in the few individuals that show signs of the disease.

In the tropical/subtropical zone of Australia the helminth parasites of greatest economic importance are *Haemonchus placei*, *Oesophagostomum radiatum* and *Cooperia* spp. Clinical disease is usually seen in dairy calves between 4 and 12 months of age and in beef calves during the first year after weaning. Adult cattle are resistant to infection and clinical signs of parasitism are unusual in animals more than 2 years old (Winks, Bremner & Barger, 1983). For these reasons, treatment with anthelmintic at weaning and for 9 months thereafter at intervals of no longer than 6 weeks is the usual recommendation for preventive control of gastrointestinal parasites in the tropical/subtropical zone.

In both climatic zones effectiveness can be enhanced and treatment frequency reduced by moving treated animals to pastures of low infectivity immediately following anthelmintic therapy. Widespread adoption of these general recommendations would be expected to confine usage of macrocyclic lactones largely to cattle aged between 9 months (weaning) and 18-24 months. Technical literature on recommended treatment programs for cattle using products based on ivermectin, moxidectin and doramectin were sought from each of the companies marketing these products in Australia. However, these were supplied in a comprehensive form only by MSD Agvet for the topical formulation of ivermectin. The parasite control programs for cattle published by MSD Agvet advocate use of the topical formulation of ivermectin in all classes of cattle, except cows where the milk is for human consumption, at various times throughout the year (see Table 8.1). In the winter rainfall zone this program would lead to minimal exposure of adult dung beetle species which breed during the spring, e.g. *O. binodis* in WA, since only calves suffering clinical parasitism would be treated at that time. However, emergence of young adults from these brood masses between December and March (Ridsdill-Smith, 1993) coincides with a time when treatment of all cattle is recommended and these immature stages are particularly vulnerable to the effects of ivermectin on ovarian development with possible impact on generation interval. In this particular environment, *O. binodis* produces a second generation each year from oviposition during February and March, so developing larvae of this generation are also confronted with a high risk of exposure to ivermectin residues in dung. Young beetles emerging from these brood masses during April and May would be feeding on dung during a period of high ivermectin usage and may incur increased levels of mortality and delayed ovarian development. In the same environment Ridsdill-Smith (1993) showed that newly-emerged *O. ferox*, which is native to south western Australia and has one generation per year, buried dung for feeding in May-June and for brood masses in which to lay eggs in October. For *O. ferox* the major risk of exposure to ivermectin residues in dung arises from the winter treatment which could increase mortality of the immature adults.

In the summer rainfall region of the temperate zone and the tropics/subtropics the recommendations for ivermectin use in Table 8.1 appear to maximise the risk of dung beetle exposure during the spring and summer when dung burial/oviposition is also high for the introduced species such as *O. gazella, E. intermedius* and *L. militaris* (Doube *et al*., 1991). Use of ivermectin in the summer rainfall and tropical/subtropical zones is also recommended for control of buffalo fly and also of cattle tick (*Boophilus microplus*) numbers during the "spring rise" of these parasites in addition to control of gastrointestinal nematodes.

Table 8.1. Recommended Cattle Parasite Control Program for Ivermectin Pour-On

Season	Zone		
	Winter Rainfall[1]	Summer Rainfall[2]	Tropics/Sub Tropics[3]
Spring (Sept/Oct/Nov)	Calves - salvage treatment	Weaners, heifers, finisher steers, bulls, breeding cows	Heifers, bulls, weaners, finisher steers
Summer (Dec/Jan/Feb)	All cattle	All cattle	-
Autumn (Mar/Apr/May)	Weaners, finisher steers, heifers, bulls	Calves - salvage treatment	Weaners, finisher steers
Winter (June/Jul/Aug)	Weaners, finisher steers, heifers	Weaners, finisher steers, heifers	All cattle

[1]Southern NSW, Victoria, South Australia, southern WA
[2]Northern NSW, southern Queensland
[3]Central Queensland, northern Australia
Source: Brochures published by Merck Sharp & Dohme (Australia) Pty Ltd.

These observations illustrate the relative degree of risk of exposure of dung beetles populations to macrocyclic lactones at different stages of the life cycle if these compounds are administered at particular times of the year to achieve optimal effect against the target parasites of cattle. However, it should not be assumed that these recommendations necessarily represent practices adopted by a majority of farmers and it is considerably more difficult to quantify the actual magnitude of risk. Forbes (1996) has attempted to do this through a reasoned analysis of 1995 quarterly sales figures for avermectin doses sold for cattle in Australia, cattle population size and composition, and dose rates for each class. Assuming a single treatment per year he calculated that a maximum of 33% of Australian cattle would have been treated once with an avermectin, or 67% would never have been treated at all. Further consideration of the relative faecal outputs of each class of cattle suggested that calves under one year old, the group of animals most likely to be treated, would produce only 12% of the dung deposited on pastures by the entire cattle population. Forbes (1996) therefore concluded that the overall exposure of non-target insects to dung from animals treated with an avermectin would be extremely low. The logic behind these calculations is not questioned but the usefulness of the conclusion derived from them is subject to serious doubt since it essentially averages out the risk across all climatic zones and rainfall regions, and across all dung beetle populations regardless of species or their relative sensitivities to the avermectins.

Without a more complex analysis of dung beetle population dynamics taking into account many factors including climatic effects, mobility, regional differences in species abundance and diversity, frequency of actual usage of macrocyclic lactones in different classes of cattle, proportion of the population exposed at particular stages of the life cycle and the relative impact of the various compounds on the different species, it is not possible to quantify potential impact on overall fitness of these beetle populations. In other words, consideration of the principal factors that regulate the rise and fall of dung beetle populations under natural conditions is needed to quantify the additional risk posed by macrocyclic lactonen usage. Unfortunately, there is still little understanding of many of these factors but progress has been made recently in developing a computer model which quantifies some of the developmental biology of *O. alexis* including characteristics of emergence, egg-laying, movement between pads and survival time (Wardhaugh & Beckmann, 1996). Although this computer model is at an early stage of development it is already possible to examine the impact of timing of treatment of livestock with anti-parasitic chemicals in relation to beetle activity. This approach may yield a more informative and reliable assessment of environmental risk associated with particular patterns of use of different macrocyclic lactones in the principal climatic zones.

8.2 Sheep

Driven by the widespread emergence of resistance to broad spectrum anthelmintics in the benzimidazole and levamisole/morantel classes, major efforts have been made since the 1980s by State Departments of Agriculture to introduce strategic worm control programs for sheep which minimise drenching frequency to strategic times of the season for maximum impact on the worm populations. Wherever possible, drenching is followed by a move of susceptible stock to 'worm safe' pasture and annual rotation of the class of broad spectrum anthelmintic used on the property between benzimidazole, levamisole/morantel and macrocyclic lactone. This rotation may be constrained by resistance to the former two classes and lead to increased frequency of macrocyclic lactone use.

In the summer rainfall region of northern New South Wales and southern Queensland, broad spectrum anthelmintic treatment is recommended for adult sheep and hoggets in August and November and for lambs and weaners in November, February and April. In the uniform (central New South Wales) and winter (southern New South Wales, Victoria, Tasmania, South Australia, Western Australia) rainfall zones, broad spectrum anthelmintic treatments are recommended for all sheep in December and February, for spring-born lambs in April and July and for autumn-born lambs in July and September. Data on actual frequency of anthelmintic usage on properties in particular districts of the summer and winter rainfall zones in 1990-1992 (see Waller, Mahon & Wardhaugh, 1993) indicated that there was a general compliance with these recommendations.

Other surveys have indicated that the 'Wormkill' program in the summer rainfall zone of New England achieved an adoption rate of 90% whereas the 'Drenchplan' program in southern New South Wales in the winter rainfall zone may not have exceeded 50% adoption rate (P J Waller, personal communication).

These seasonal patterns of anthelmintic application indicate that dung beetles are potentially exposed to sheep faeces containing residues of macrocyclic lactones on several occasions throughout their active feeding and dung burial periods in spring, summer and early autumn. Exposure risk appears highest when classes of sheep receive broad spectrum treatment against gastrointestinal parasites during the late spring/mid-summer period in the summer rainfall zone and during the early- and mid-summer period in the winter rainfall zone. The population dynamics, seasonal abundance of different species, and biology of beetles utilising sheep dung as a resource are even less studied than those of cattle dung so it is not possible to quantify the likely impact of macrocyclic lactone treatment of sheep on these populations. Nevertheless it is expected to be extremely low overall given that avermectin residues in sheep faeces appear to be toxic to developing larvae for a maximum of 5 days after drenching in the two species of beetles that have been examined. The risk factor is lowered further by the probability that macrocyclic lactones should be used only one year in three, but the high prevalence of resistance of sheep parasites to the other two classes of broad spectrum anthelmintics suggests that actual usage of macrocyclic lactones on sheep properties could be substantially higher, perhaps as high as 50% in some districts (see Waller *et al*., 1993). However, analysis of the total Australian sheep drench market figures for 1993 indicated that 28% of doses sold were in the macrocyclic lactone class (G Davies, personal communication). No data have been submitted to indicate whether the overall proportion of sheep drench market shared by macrocyclic lactones has changed since 1993. Entry of moxidectin into the marketplace since then may have displaced some of ivermectin's share thereby maintaining the same risk exposure to dung beetle populations. However, such competition seems more likely to have increased the overall proportion of sheep being dosed with a macrocyclic lactone and therefore the exposure of dung beetle populations.

It is immediately apparent that the intraruminal controlled release bolus for sheep which delivers daily ivermectin treatment at 20 ug/kg/day for 100 days substantially increases the period of exposure to ivermectin residues by those beetles which use sheep dung as a resource. However, there are no data on which to base any quantitative judgement of exposure risk except by extrapolation from studies of toxicity of sheep dung following single dose ivermectin therapy which have been done in only two species. One of these studies suggests that levels of ivermectin found in sheep dung up to 5 days after treatment may cause high mortality in the developing larvae of some *Aphodius* spp. Measurements by Ali (1994) in pen-fed sheep showed that between the 3rd and 4th day after ivermectin treatment at 200 ug/kg approximately 10% of the dose was excreted in faeces, that is, the equivalent of 20 ug/kg.

On reaching equilibrium during treatment with the sustained release bolus daily excretion of ivermectin residues should approximate the dose rate of 20 ug/kg. The introduced dung beetle *E. fulvus* may be less at risk since toxic effects on developing larvae and immature adults were apparent only in faeces excreted between 1 and 2 days after oral treatment with ivermectin. At least 40% of the single 200 ug/kg dose is excreted in faeces during this period (Ali, 1994), which is equivalent to four times the expected faecal excretion of sheep given the intraruminal bolus. Nevertheless, there is clearly a need to assess the effects of long-term exposure of beetle populations to dung of sheep receiving the ivermectin bolus because of the possibility of toxic but sub-lethal effects at low concentrations.

8.3 Horses

The manufacturer's recommendations for the use of ivermectin formulations (Equimec®) in horses suggest that all horses be included in a regular parasite control programme with particular attention to mares, foals and yearlings. It is advised that foals receive initial treatment at 6 to 8 weeks of age and routine treatment be repeated as appropriate for the property with the frequency being assessed by a veterinarian taking account of stocking rate, age distribution, grazing access, climate and parasite infestation status of the property. Traditionally, control of horse parasites in Australia has recommended routine anthelmintic treatments at 6-8 week intervals but more recently strategic worm control programs have been advocated which integrate pasture hygiene with anthelmintic treatment and annual rotation of chemical class between macrocyclic lactone and benzimidazole (Huntington *et al.*, 1993). Under these programs ivermectin treatment would be given to yearlings and 2-year-olds in November, January, March and July, mares in September, November, March and July, and adult horses under high stocking rates in November, January and July. These timings clearly coincide with periods of dung beetle breeding and emergence and must therefore potentially expose populations to toxic residue levels.

Obviously, use patterns could vary enormously between horse properties and no data on usage have been submitted to this review to indicate the actual risk exposure of dung beetle populations on horse properties. The horse industry tends to be highly aggregated regionally so that regular ivermectin treatment on adjoining properties could generate a high risk exposure to dung beetle populations in a particular district if all horses were grazed. Certainly indigenous species are as attracted to horse faeces as other herbivore dung (Doube & Wardhaugh, 1991) but there are no data available on the influence of ivermectin treatment of horses on dung colonisation or mortality of these species under Australian conditions. The very rapid rate of faecal excretion of ivermectin over the first 2-3 days after dosing generates concentrations that are known to cause high mortality and inhibit development of the larvae of many species of dung beetles. However, this rapid clearance and consequent short duration of exposure to ivermectin residues in horse dung suggests that overall impact on beetle populations would be low.

8.4 DOGS

Monthly prophylactic treatment of dogs with ivermectin tablets is recommended to prevent heartworm disease. The low dose rate (6 ug/kg) and rapid elimination in faeces (t ½ = 1.8

days; Fink & Porras, 1989) indicates an extremely low exposure risk to dung beetles using dog faeces as a food resource. Similarly, monthly treatment of dogs with moxidectin tablets at a dosage of 3 ug/kg would pose an even lower risk to dung beetles. Milbemycin oxime tablets are prescribed for prevention of heartworm disease and control of roundworm and hookworm infections at a higher dosage of 0.5 mg/kg monthly but, in the absence of information on toxicity to dung beetles, it is not possible to assess whether this compound represents a significant risk to beetle populations. It seems improbable that, even in an urban environment, dog faeces would represent more than a minor food resource for indigenous dung beetle populations and monthly treatment of dogs with any of these macrocyclic lactones seems irrelevant to population sustainability. The increasing trend towards making urban dog-owners responsible for cleaning up public areas fouled by their pets reduces the exposure risk for dung beetles even further.

9. INFLUENCE OF DIET AND MANAGEMENT PRACTICES ON MACROCYCLIC LACTONE AVAILABILITY TO DUNG BEETLES

There is very little published information to indicate the influence of dietary factors on macrocyclic lactone excretion in faeces. Cattle grazing on irrigated pasture and receiving a hay supplement had lower faecal concentrations of ivermectin following subcutaneous treatment than animals housed in a feedlot and given hay:lupin grain (40:60) ration (Cook *et al*., 1996). Peak concentrations were respectively 360 and 90 ppb wet weight at 6 and 8 days post injection on the grain and pasture diets but were similar on both diets by day 14. Nitrogen content was lower and pH higher in the faeces of the pasture-fed compared to the grain-fed cattle. In cattle and buffalo systemic availability of fenbendazole was lower in animals feeding on green fodder than on a poor quality, dry hay diet (Sanyal *et al*., 1995); it was suggested that decreased transit time of digesta on green diets may reduce time available for gastrointestinal absorption of drug. It is well established that as diet quality improves both digestibility and intake increase and the residence time of digesta in the gastrointestinal tract decreases. Comparisons of dietary effects on plasma concentrations in lambs dosed orally with ivermectin showed lower peak levels and systemic availability in grazing animals compared to those fed hay and concentrates (Taylor *et al*., 1992) which may have been associated with a shorter residence time of digesta in the gastrointestinal tract. Since feed intake is a major determinant of the rate of passage of digesta in ruminants given forage diets it might be expected to influence the rate of appearance and level of ivermectin residues in faeces. Although Ali & Hennessy (1996) found that reducing feed intake of sheep extended the residence time and increased levels of ivermectin in the rumen and abomasum, the rate of appearance and total recovery of drug residues in faeces were not affected.

It is difficult to draw any general conclusions from these observations about the dietary and gastrointestinal factors that determine faecal excretion levels and profile following ivermectin therapy in cattle or sheep. Cook *et al*. (1996) showed that profound differences in faecal residue levels can occur between diets differing markedly in their physical and chemical composition by inclusion of grain. These findings suggest that supplementary feeding with grain of cattle at times of ivermectin treatment may heighten the toxicity of faeces and accentuate any negative impact on dung beetle populations. Information is not available to assess the likely

frequency of such occurrences but it is probably extremely low. Risk also occurs in feedlots which use grain-based diets and where cattle are routinely treated for internal parasites on entry. Disposal of the faeces of these animals on pastureland surrounding the feedlot may expose dung beetle populations to macrocyclic lactone residues. Again, the potential impact is difficult to quantify without data on treatment frequency and dung disposal methods at particular feedlots. Assuming a single treatment of cattle at 200 ug/kg, Environment Australia has estimated exposure following spreading of manure under a worst case scenario that assumes a single treatment of cattle at 200 ug/kg (entry weight 270kg) followed by lot feeding for 70 days, during which time wet manure production in the order of 1400 kg would be expected. Assuming that scraped manure removed from the pens after lot feeding contains 30% water (compared with 90% for wet manure) and has lost 30% of its organic matter to decomposition, the residue concentration in scraped manure approximates 100 ug/kg. Spreading at a rate of 40 tonnes/ha provides an estimated residue concentration in 15 cm soil, density 1.2, of 2.3 ug/kg. Noting the worst case assumptions (no metabolism in the animal or degradation in manure) and the predilection of dung beetles for fresh dung, risk to overall dung beetle populations from spreading of feedlot manure appears low.

10. CONCLUSIONS

Although the intent of this review was to examine the macrocyclic lactones as a class of chemical it has revealed that there are differences in both level of information and documented effects between the various constituent compounds of this class which are marketed as formulated products in Australia. There are substantially more published papers in scientific journals on ivermectin and abamectin effects on dung insects and dung degradation than for moxidectin and there are none on doramectin. It is therefore appropriate to present separately for each of the compounds the major conclusions about known effects of ivermectin/abamectin, moxidectin and doramectin on dung beetles and dung degradation under Australian conditions.

10.1 Ivermectin/Abamectin

10.1.1 Cattle

Administration of these compounds to cattle in subcutaneous or topical formulation has the following effects on a range of introduced dung beetle species:

- no toxicity to mature egg-laying adults
- increased mortality and impaired development of larvae with sub-lethal effects on the morphology of some species. These effects are mostly confined to dung voided within 2-3 weeks of treatment.
- increased mortality and delayed reproductive development in newly emerged adults of some species feeding on dung voided within 1 to 2 weeks of treatment.

Dung of treated cattle is highly toxic to dipteran larvae, inhibiting development for periods ranging from 2 to 8 weeks.

The duration of these toxic effects on dung insects is consistent with the profile of excretion of these avermectins in cattle faeces.

Under European field conditions, treatment subcutaneously or topically with these compounds has little effect on colonisation of cattle dung pats by adult coprophagous beetles but larval numbers are reduced in dung deposited for up to 2-3 weeks after treatment. Dipteran larvae are reduced in dung pats for up to 4 weeks after treatment. No comparable long-term studies have been undertaken under Australian conditions but there is evidence that adult native and introduced species of dung beetles actively colonise, and may be more attracted to, the dung of treated cattle.

Under European field conditions there is no evidence for long-term adverse effects of ivermectin or abamectin residues on the degradation of dung pats or on the accumulation of dung on pasture, despite initial reductions in insect colonisation and dung disappearance rate. No comparable long-term studies have been undertaken under Australian conditions where short-term increases have been recorded in burial rates of dung containing avermectin residues.

Usage patterns of ivermectin and abamectin products in cattle expose dung beetle populations to risk from toxic residues during larval development and following emergence of immature adults. The level of risk may be altered by dietary influences at the time of treatment, such as grain feeding, but the critical dietary determinants of faecal residue levels are poorly understood.

Quantitation of the overall impact of ivermectin and abamectin products for cattle on the population dynamics of native and introduced dung beetles under Australian conditions is not possible from the present state of knowledge. The development of computer models to simulate the biological and physical determinants of the population characteristics of the introduced dung beetle species should assist in identifying the most sensitive elements of risk to these populations and enable livestock treatments to be timed optimally to minimise that risk whilst maintaining a balance between effective parasite control and sustainable beetle populations.

10.1.2 Sheep

Limited studies on only one species of introduced dung beetle show that oral administration of ivermectin to sheep has no effect on adult mortality, has short-term effects on development of larvae, and increases mortality and impairs reproductive development of newly emerged adults in dung voided 1-2 days after treatment. These short-term effects are consistent with the rapid excretion of residues in dung, and indicate that other species feeding on sheep dung will be exposed to toxic residues for a short time only.

The recommended use pattern for oral ivermectin drenches in sheep in the summer and winter rainfall zones indicates that native and introduced dung beetle populations will be exposed to ivermectin residues periodically throughout the active breeding cycle. However, the short-term toxicity of these residues suggests that there is a very low risk to sustainability of these populations.

No data have been submitted to enable assessment of the toxicity to dung beetles of faeces from sheep treated with ivermectin continuously for 100 days using an intraruminal bolus, which substantially increases the exposure risk for both native and introduced species. Calculated residue levels in faeces during bolus therapy suggest that these may be below the toxic threshold for introduced dung beetles. Faecal accumulation was not affected on paddocks housing sheep during and after treatment with the ivermectin controlled release bolus, but this does not adequately measure impact on populations of dung beetles because of their minor role in decomposition of sheep dung under Australian conditions (see King 1993) although they undoubtedly use it as a feed resource. Sub-lethal effects on dung beetles of long-term exposure to these low ivermectin residue levels in sheep dung have also not been evaluated and are necessary to assess the potential for impact on population sustainability.

10.2 Moxidectin

10.2.1 Cattle

Limited studies indicated that moxidectin residues in cattle dung are not toxic to egg-laying adults or to developing larvae of two species of introduced dung beetles. A short-term field trial under European conditions showed that colonisation and larval development of native Coleoptera in dung pats were unaffected by moxidectin residues.

Development of dipteran larvae of *Musca* spp. is not affected by moxidectin residues in cattle dung. Duration of impaired larval development of *Haematobia irritans* varies widely from 3-28 days following treatment.

No data have been published or submitted by registrants on the effects of moxidectin residues in cattle dung on mortality of newly emerged adult dung beetles or their reproductive development.

No field studies have been reported under Australian conditions of the impact of moxidectin treatment on dung colonisation by native and introduced beetles or on dung degradation. Moxidectin is apparently less toxic intrinsically to larvae of one species of introduced dung beetle and to buffalo fly than abamectin.

Slower rates of excretion in faeces presumably contribute to the lack of toxic effects on larval development but there has been no examination of possible sublethal effects on beetle populations arising from the longer term excretion pattern of moxidectin in cattle dung. Although the limited data available might suggest that moxidectin appears unlikely to have a negative impact on dung beetle populations, there is clearly a need for more extensive and longer-term testing against a wider range of introduced species to adequately define the risk.

10.2.2 Sheep

Residues excreted in sheep dung during the first two days after oral treatment reduce the development of larvae of the one species of dung beetle which has been examined. This is consistent with the high residue levels in faeces at that time and suggests that other species may be similarly affected. These observations together with the protracted excretion profile for moxidectin residues in sheep dung indicate the need to evaluate possible sub-lethal effects on larvae and immature adults of the more important Australian species in order to assess the risk exposure to dung beetle populations of moxidectin drenching of sheep.

The one study which has been submitted of toxicity of sheep dung residues to Diptera indicates that larval development may be affected for only two days after an oral treatment with moxidectin.

10.3 Doramectin

10.3.1 Cattle

There are no data available to indicate whether doramectin residues in the dung of treated cattle affect any stage of the life cycle of coprophagous beetles. Laboratory experiments have established an LC_{90} for doramectin in cattle dung against the larvae of one introduced species. Pharmacokinetic data indicate that this concentration is exceeded in cattle faeces for at least 2 weeks after treatment suggesting that doramectin may have toxic effects on dung beetles when used in cattle.

No data have been presented of the effects of doramectin residues on colonisation of dung by insects or on dung degradation which are relevant to Australian conditions.

10.3.2 General

This review of currently available data published in the scientific literature and submitted 'in-house' company reports has shown that there is a need to generate further information to more adequately quantify the risk to dung beetle populations posed by specific compounds within the macrocyclic lactone class.

A detailed analysis of the risk to the more important introduced species of coprophagous beetles associated with use of ivermectin and abamectin e products in cattle in Australia utilising all relevant information on their toxic effects would increase understanding of potential to affect dung beetles and dung degradation. This should take into account regional distribution and population dynamics of the species and optimal use pattern of the pour-on and subcutaneous formulations to achieve control of target parasites. In the first instance the computer model being developed for *O. alexis* could be a useful tool since use of these avermectins seems to occur primarily in New South Wales and Victoria (see Forbes, 1996) where this species is abundant. It may be appropriate to validate model predictions by limited field trials with the emphasis on measuring impact on population dynamics.

To establish the influence of long-term exposure of dung beetles to residues in faeces during treatment of sheep with the ivermectin controlled release bolus, further data on effects on representative dung beetles species are needed. Species and feeding pattern should be representative of the field situation in the summer and winter rainfall zones.

Further investigation of toxicity of moxidectin residues in cattle and sheep faeces to other genera of introduced dung beetles, particularly *Onitis* spp., is necessary to understand the risk to dung beetles and dung degradation from use of this compound. Sublethal effects of moxidectin residues on larval development, on mortality of newly emerged adults, and on their reproductive development should be evaluated in the more important introduced genera of Australian dung beetles.

If the latter studies indicate toxic effects on all or some species it would be appropriate to model the impact of moxidectin treatment on dung beetle populations as suggested for ivermectin/abamectin products.

A complete data package is required of the short- and long-term lethal and sub-lethal effects of doramectin residues in cattle dung on the more important introduced species of coprophagous beetles. This should enable an initial assessment of the risk to dung beetles from use of doramectin products in cattle on which decisions would be based whether to proceed with modelling the likely impact on dung beetle populations.

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